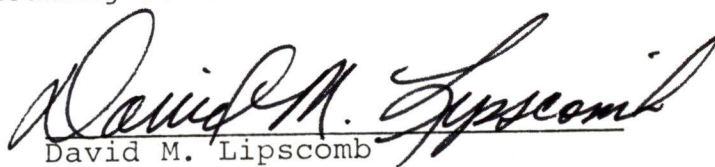
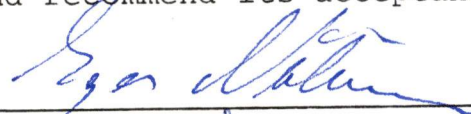
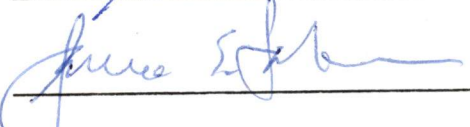
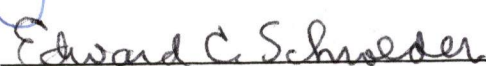


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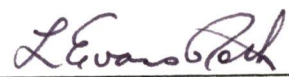
I am submitting herewith a dissertation written by Gary D. Harris entitled "Auditory Damage Following Noise Over-Exposure and Carbon Dioxide Inhalation in Chinchilla." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Speech and Hearing Science.


David M. Lipscomb
Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:


Vice Chancellor
Graduate Studies and Research

AUDITORY DAMAGE FOLLOWING NOISE OVER-EXPOSURE
AND CARBON DIOXIDE INHALATION
IN CHINCHILLA

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Gary D. Harris

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ABSTRACT

Fifteen chinchilla were exposed to a 123-dB SPL narrow band noise for 50 minutes. Immediately following noise exposure they were placed in an atmosphere of either Carbogen (5% CO₂/95% O₂), Carmal (5% CO₂/21% O₂/74% N₂), or air for 20 minutes. Each of the three groups contained five chinchilla and the average preexposure auditory brainstem electrical response (ABER) thresholds of each group were approximately equal.

Twenty-six days following noise exposure the experimenter, without knowing which gas mixture the animals received, established ABER thresholds to tone bursts, prepared the cochleae for histologic study, and conducted hair cell counts to establish the extent of noise-induced cochlear damage.

The air breathing group suffered the greatest electrophysiologic threshold shift and total hair cell loss, but the differences among groups were not statistically significant. There were however trends in the histologic results which suggest that a mixture high in CO₂ may decrease the amount of postnoise exposure auditory damage in the apical part of the cochlea. The results are compared to those of other studies which report Carbogen to reduce noise-induced auditory damage.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Acoustic over-stimulation damages the auditory system and causes noise-induced hearing loss (NIHL). NIHL typically always has a temporary component and often a permanent component. The fact that temporary threshold shift (TTS) exists following acoustic over-stimulation implies that the auditory system changes function temporarily; and the amount by which the function changes could contribute to the amount of permanent threshold shift (PTS) which remains after TTS.

The purpose of this literature review is, in part, to acquaint the reader with the feasibility of intervening and temporarily altering the auditory system's physiologic environment, and by doing so decreasing noise-induced TTS and PTS. The various attempts to alter NIHL through physiologic means will also be discussed.

In order for a method of aiding recovery from NIHL to be feasible, some of the mechanisms by which the auditory system responds to excessive noise must be altered.

One of the mechanisms of NIHL is that of mechanical damage to the organ of Corti (Spoendlin, 1971; Spoendlin and Brun, 1973; Spoendlin, 1976; Bohne, 1976; Lipscomb et al., 1977; Durrant, 1978). Mechanical damage implies that the motion of the basilar membrane, during high level stimulation,

produces enough stress to cause the organ of Corti and its attachments to rupture (Bohne, 1976) with tearing and dislocation of cochlear tissues (Lipscomb et al., 1977). Mechanical damage as defined here is permanent and does not seem to be a mechanism which could be altered; except perhaps by altering other mechanisms which might contribute to the ultimate mechanical damage (e.g., metabolic insult).

Examination of noise-exposed cochleae has shown evidence of other factors involved in auditory damage other than from mechanical damage alone. These factors are classified as metabolic and include:

- Endolymphatic oxygen decreases (Misrahy, 1958),
- Inner ear glycogen decreases (Ishii et al., 1969),
- Altered ionic exchange between the scalae (Suga et al., 1970; Nakashima et al., 1970; Bohne, 1976),
- Swelling of afferent dendrites (Spoendlin, 1971; 1976),
- Swelling of afferent dendritic mitochondria (Lim and Melnick, 1971),
- Perilymphatic lactate increases (Schnieder, 1974),
and
- Swelling of outer hair cell nuclei (Spoendlin, 1976).

The endolymph of the scala media is normally twenty to thirty times higher in potassium and two to five times lower in sodium concentration than perilymph (Chou and Rodgers, 1962). These large ionic differences, as well as large electrical potential differences [as much as 224 mV between

the organ of Corti and stria vascularis (Honrubia et al., 1976)], suggest that the cochlea is metabolically very active.

With excessive noise however these ionic differences decrease; that is, the endolymphatic potassium decreases and sodium increases, while the perilymph of the scala vestibuli shows decreases of sodium and increases of potassium (Suga et al., 1970; Nakashima et al., 1970). This suggests that, during over-stimulation, the permeability of Reissner's membrane is increased and/or the ability of the sodium potassium pump to maintain and produce ionic differences is hampered.

More importantly to the purposes of this study is that asphyxia (produced in animals by tracheal cannula occlusion) without sound stimulation produces nearly identical results (Suga et al., 1970). That is, endolymphatic increase in sodium and decrease in potassium accompany both asphyxia and noise exposure. This suggests that noise may cause a decrease in oxygen, which in turn may be responsible for altered ionic exchange during over-stimulation. Another indirect basis for suggesting that noise may induce cochlear hypoxia is the decrease in glycogen and increased reliance on anaerobic glycolysis in the cochlea during sound over-exposure (Ishii et al., 1970).

Schnieder (1974) also provided indirect support of the importance of oxygen in the pathogenesis of NIHL in his

report that the lactate of the perilymph increases after exposure to noise. (Lactate is the terminal product of anaerobic glycolysis.)

Direct support that noise causes cochlear hypoxia is given by Misrahy et al. (1958), who measured endolymphatic oxygen availability in guinea pigs. A ten to fifteen second pure-tone burst of 130 to 135 dB SPL produced an artifactual (sic) increase in oxygen tension. The cessation of sound was followed by a drop in endolymphatic oxygen availability of 20 to 90%, returning to normal value only after twenty minutes.

The importance of oxygen to the cochlear microphonic (CM) was suggested by Lawrence et al. (1975). They measured CM and endolymph and cortilymph oxygen content in guinea pigs under two conditions: with normal air to the animal, and with no air to the animal. The CM declined at the same time as the cortilymph oxygen content declined. They reported that the CM is partially dependant upon oxygen supplied to the extracellular spaces of the organ of Corti by the spiral vessels.

Tonndorf et al. (1955) studied the combined effect of sound stimulation and oxygen deprivation on the CM in guinea pig. They recorded gradient curves of cochlear microphonics during and after various combinations of noise exposure (1 kHz tone at 130-dB SPL for 1 minute) and oxygen deprivation

(10% oxygen/90% nitrogen; and 8% oxygen/92% nitrogen). Their results indicate that the CM is moderately depressed by oxygen deprivation, but that the amount of depression depends upon the degree of oxygen deprivation in combination with the magnitude of the simultaneous noise exposure. When normal air (20% oxygen/80% nitrogen) was restored after sound exposure, the rate of CM recovery was faster than after sound exposure and continued oxygen deprivation.

These studies have indicated the importance of oxygen to cochlear potentials. One group of investigators however have questioned this (Butler et al., 1962). They perfused scala vestibuli with Ringer's solution immediately prior to onset of asphyxia, with perfusion being continued during the asphyxic period. The decline of cochlear potentials was markedly retarded and CM remained well above the level normally observed during oxygen deprivation. One explanation was that the perfusate is washing away some toxic agent that accumulates in scala media during asphyxia. They point out however that the Ringer's solution may have been supplying some oxygen to the organ.

Dallos (1973) reported that with total lack of oxygen (after death) there is an initial precipitous drop of the CM, followed by a very slow decrease in the residual CM. He suggested that the component that decreases immediately upon expiration is of a different origin than the component that

lingers on. The two components are often designated as aerobic and anaerobic, respectively. In contrast to the CM and other cochlear potentials, the VIIIth nerve compound action potential (CAP) disappears completely and abruptly with total anoxia.

Johnstone (1975) suggested that the hair cells themselves were relatively resistant to lack of oxygen; that the nerve terminals (the dendrites under the hair cells) are more sensitive to oxygen lack. He had guinea pigs breathe a 5% oxygen/95% nitrogen mix and measured the action potential and single unit nerve fiber response. Nothing happened for a couple of minutes but then the action potential failed and the single unit fiber lost about 30 to 40 dB sensitivity. When the animal was allowed to breathe normal air again the action potential was restored.

Based on the literature reviewed it now seems warranted to conclude:

1. Normal oxygen supply is important to both cochlear and neural function, perhaps neural function to a greater extent;
2. CM recovery is slower than usual when too little oxygen is available for inhalation;
3. Acoustic over-stimulation causes a relative lack of oxygen in the cochlea which does not return to normal immediately after cessation of noise;

4. Noise induced hypoxia may cause other metabolic disturbances such as altered ionic exchange;
5. Acoustic over-stimulation may cause a build up of toxins within the cochlea.

Two of the possible explanations for oxygen lack during and following noise exposure include: increased oxygen consumption as a result of increased stimulation and, oxygen lack as a result of noise-induced vasoconstriction to vessels supplying the auditory system. These two factors are not mutually exclusive and may exist simultaneously. However, the literature concerned with the role of blood circulation in the pathogenesis of NIHL takes two opposing views.

In 1961 Perlman and Kimura reported that noise exposure had little or no effect on the vessels of the cochlea. They fenestrated and transilluminated the apical cochlea of guinea pigs, viewing the stria vascularis and spiral ligament vessels. Sound more intense than 90 dB was found to cause no change or slight increase in flow. To stimulation as intense as 153 dB however the authors noted: ". . . flow in an occasional vessel appears to stop." Most noise stimulation is not as great as 153 dB so that for most intensities blood flow is not altered. Criticizing Perlman and Kimura's study, Schnieder (1974) pointed out that only a portion of the cochlear vessels was viewed, and that blood flow may differ considerably in the various sections of the spiral ligament.

He further argued that the flow velocity of erythrocytes, on which Perlman and Kimura based their study, does not strictly correlate with cochlear blood flow.

Morimitsu et al. (1965) used electrical impedance plethysmography as an indirect measure of cochlear blood flow. They found that stimulation with a 1 kHz tone of 120 phone (sic) for 10 minutes produced no measurable change in cochlear blood flow. Impedance plethysmography has been widely used by Suga and associates (1969a; 1969b; 1969c; Snow and Suga, 1973) to measure cochlear blood flow response to various vasodilators and appears to be a valid method for measuring blood flow changes in the cochlea. Although the Morimitsu et al. study has not been replicated, their findings argue strongly against the hypothesis that vasoconstriction is important in the pathogenesis of NIHL. Several other studies however, using other methods, have provided contrary evidence.

In a histologic study, Kellerhals (1972) found that in guinea pigs exposed to gunshots the capillaries of the stria vascularis were densely packed with red blood cells. The capillaries appeared to be filled with an almost homogenous mass, whereas in control animals they were loosely filled with erythrocytes. This observation could be made immediately following, or several days after noise exposure. While his results do not necessarily imply vasoconstriction, they do suggest that microcirculation is altered by excessive noise.

Hawkins et al. (1972) stimulated guinea pigs with a wide band noise of 120-dB SPL for thirty hours. Histologically they found evidence of vasoconstriction in the outer spiral vessels with multiple narrowings of the lumen of the vessel. The narrowing suggests the participation of swollen endothelial cells (cells lining the vessel) and pericytes (an elongated cell found wrapped around capillaries) in impeding the movement of the oxygen carrying erythrocytes.

In another histologic study, Duvall et al. (1974) exposed animals to a 123 dB noise for fifteen minutes. Five to ten minutes postexposure the stria vascularis appeared normal. One hour after exposure they observed that some vessel loops (under the organ of Corti) had collapsed. By three hours after exposure many vessels of the stria were collapsed, and then by seven days stria repair was evident. They concluded that following noise there are temporary but significant structural changes in the stria vascularis and that permanent changes occur in the vessels of the spiral prominence. This conclusion, however, may be valid only when considering the given stimulus intensity and duration.

To assess cochlear damage due to ischemia, Bohne (1976) terminated chinchilla and placed the cochleae in a nitrogen atmosphere before fixating. After two hours of ischemic conditions the inner hair cells were ruptured while the

plasma membranes of the outer hair cells were still intact. She concluded that, since the outer hair cells are typically the first cells damaged by noise, lack of oxygen during exposure to noise is not likely to cause sensory cell damage. This conclusion seems ill-based in that the results were obtained with total lack of oxygen—after death—and without noise exposure to the animal. Postmortem degeneration is not necessarily identical to that caused by excessive sound. Also, noise over-exposure causes a relative lack of oxygen in combination with mechanical stress.

Bohne (1976) also studied the role of cochlear vascular change due to noise. She exposed animals to noise and then terminated the animals as soon as possible (but no sooner than twenty minutes) after exposure. Some animals were exposed to a 500 Hz octave band noise of 95-dB SPL for nine days and others to a 4000 Hz noise of 100-dB SPL for three and a half hours. The conditions of the vessels under the damaged portions of the organ of Corti were observed only. With few exceptions, no abnormalities were noted in these vessels.

Lipscomb et al. (1977) have provided histologic evidence contrary to that of Bohne. They stimulated chinchilla for eight hours with a 110-dB SPL wide band noise. The surface cells of the stria vascularis appeared uneven, swollen or shrunken, and with intracellular gaps. Another common

finding was the appearance of vacuoles in the stria vascularis.

Perhaps the most extensive histologic study of the vascular effects of noise exposure in the chinchilla cochlea has been that of Vertes et al. (1979). One major asset of this study is that noise-exposed and control animals were mixed and randomly assessed without prior knowledge of the group to which the specimen belonged. Chinchilla were exposed to pink noise at levels ranging from 110 dB for 8 hours to 125 dB for 15 minutes. They reported that three weeks after exposure significant differences between exposed and control animals were found. Red blood cells were less frequent and spaced further apart in the radiating arterioles and collecting venules of the spiral lamina and in the limbus vessels of exposed animals. Several other vascular parameters were disturbed or more variable in the exposed animals. However, some parameters previously considered to be typical examples of vascular degeneration, such as perivascular spaces and avascular channels, were found equally often in noise-exposed and control animals. The vascular changes found were more frequent in the apex than the base of the cochlea and did not necessarily coincide with the location of sensory cell damage (which was only slight).

While there is wide support for the notion of noise-induced cochlear hypoxia, the role of cochlear microcirculation is less clear. Most histologic studies have reported

microcirculatory disturbances following noise but investigators using other techniques have found little change in cochlear blood flow in response to stimulation. The more recent of the reviewed studies have indicated cochlear blood circulation is indeed altered after acoustic over-stimulation, however. Further, those vascular changes that do occur may even be permanent.

This notwithstanding however, a logical means of altering the course of NIHL would be to improve the oxygenation of the cochlea and/or improve the removal of toxins during or after noise exposure. This in turn might be accomplished by providing hyperoxygenated blood to the cochlea and/or increasing blood flow into and out of the cochlea. Several investigators have attempted to do this with some degree of success. Others have attempted to alter NIHL through the use of substances other than those which would alter cochlear blood flow and/or oxygenation. These later substances include:

- Vitamin A [Willemse, 1952 (as referenced in Ward and Glorig, 1960); Ruedi, 1954];
- Atarax, meprobamate, luminal, caffeine (Wojnarowska, 1966);
- Adenosine triphosphate, Cytochrome C (Jankowski, 1974);
- Amino-oxyacetic acid (AOAA) (Bobbin et al., 1976).

Ward and Dixon (1960) summarized studies purporting vitamin A therapy for the improvement of hearing sensitivity and reduction of noise-induced TTS (NITTS). They also had subjects ingest either placebo or vitamin A and measured TTS. Vitamin A had no effect on NITTS although they pointed out that a vitamin A deficiency might hamper recovery from NITTS.

Wojnarowska (1966) reported that several substances were capable of altering NITTS. Of these substances the most significant results were obtained with atarax. In a partial replication of Wojnarowska's study, White and Ward (1969) gave subjects either atarax or placebo and compared pre- and postexposure thresholds. Atarax or placebo had no effect on either the amount or duration of auditory fatigue.

Jankowski (1974) reported that the prophylactic administration of Cytochrome C (which promotes the oxidative process), adenosine triphosphate (which promotes the energetic process) or both had the effect of reducing the loss of CM following exposures lasting five, fifteen and thirty minutes. They were less advantageous in terms of the compound action potential (CAP) loss however. The specifics of Jankowski's procedures were not reported.

The endocochlear potential is a resting potential necessary for cochlear function, and may play an integral role in NIHL (Bobbin et al., 1976). To test whether

reduction in the endocochlear potential could be correlated with a reduction in noise-incuded hair cell loss, Bobbin et al. (1976) used amino-oxyacetic acid (AOAA), which has been shown to reversibly decrease the endocochlear potential. Pretreatment of guinea pigs with AOAA was found to reduce the incidence of hair cell loss due to noise exposure. It was hypothesized that this occurred because a reduction in the endocochlear potential prevented the depletion of adenosine triphosphate.

The effect of adenosine triphosphate, Cytochrome C, and AOAA on NIHL appears to be a promising research area, and replication of the Jankowski and Bobbin et al. studies is needed. The present study, however, was not directly concerned with these agents.

In attempts to alter NIHL, other investigators have used substances which might improve cochlear blood flow and/or oxygen supply. These agents include:

- Dextran 40 (Kellerhals, 1971; 1972);
- Oxygen inhalation (Joglekar et al., 1977);
- Carbogen inhalation (Joglekar et al., 1977;

Witter et al., 1980; Harris, 1979; Brown, 1980).

Reportedly, low molecular weight dextran (dextran 40) improves capillary blood flow and inhibits the aggregation of blood cells and other blood corpuscles (Kellerhals, 1972). Kellerhals et al. (1971) studied ten workers exposed to the

noise blast of an explosion. Five of the workers were treated with dextran 40, and the others were left without treatment. Recovery from inner ear damage, as measured by audiometric bone conduction thresholds, proved significantly better in the treated group. This result supports the concept that microcirculatory disturbances play an important role in the pathogenesis of NIHL.

It also suggests a means whereby NIPTS might be reduced if administered soon enough after acoustic trauma occurs. However, the administration of dextran 40 should not be done without due precaution. Some of its potential side-effects include renal failure, bleeding (prolonged prothrombin time), and others (Lewis, 1975). It is also recommended that central venous pressure be monitored during the initial infusion of the drug (Lewis, 1975). Possibly for these reasons, the use of dextran 40 in cases of NIHL has not been widespread.

Although acoustic over-stimulation causes cochlear hypoxia, the use of pure oxygen inhalation has been shown not to have a large effect on the course of NITTS (Joglekar et al., 1977). The reasons may best be illustrated by referring to the work of Snow and Suga (1973), and Hou and Lipscomb (1978).

Snow and Suga (1973) found that pure oxygen inhalation decreased cochlear blood flow by as much as 70%.

Hou and Lipscomb studied the effects of 100% oxygen inhalation (hyperoxia) on the CAP in chinchilla. In response to brief (ten minutes or less) hyperoxia, there was a depression of the CAP amplitude which changed in a rapid and reversible manner. Further, a sudden flush of pure oxygen into the breathing chamber selectively removed the neural component (CAP) but left the CM unchanged, an effect similar to the results of hypoxia. This CAP depression to brief hyperoxia was attributed to a sympathetic reflex that constricts cochlear blood vessels.

Repeated or prolonged hyperoxygenation (five hours of oxygen inhalation) reduced the CAP amplitude, lengthened the latency and altered the waveform. These effects were reported as being permanent and due to oxygen toxicity (Hou and Lipscomb, 1978).

Schneider (1974) suggested that respiration of a mixture with an elevated carbon dioxide content might be useful in altering recovery from noise damage. Carbon dioxide is a powerful cochlear vasodilator (Suga and Snow, 1969c; Snow and Suga, 1973; Pollack et al., 1974; Fisch et al., 1976).

Snow and Suga (1973) observed that inhalation of a 10% carbon dioxide/90% oxygen mix increased cochlear blood flow by 244% after ten minutes of inhalation. Pollack et al. (1974) also noted that maximum otic blood flow could be obtained with a gas of 10% carbon dioxide/90% oxygen.

Fisch et al. (1976) reported that carbon dioxide produced the most marked increase of oxygen tension in human perilymph when compared to other vasodilators and maximum oxygenation occurs after thirty minutes of 5% carbon dioxide/95% oxygen respiration. No change in the perilymphatic oxygen tension was noted after administration of dextran 40. Further, a gas mixture of 5% carbon dioxide/95% oxygen can be inhaled by humans without side-effect, although caution should be exercised before administering higher concentrations of carbon dioxide than 5% (Fisch et al., 1976).

Joglekar et al. (1977) were the first to report a decrease in NIHL as a result of 5% carbon dioxide/95% oxygen (Carbogen) inhalation. Human subjects were exposed to a 110-dB SPL, 1 kHz pure tone for ten minutes. First, the subjects were exposed to the tone while breathing ambient air and the hearing shift measured and recovery monitored. After complete recovery, the subject was again exposed while breathing pure oxygen. Finally, subjects breathed Carbogen during exposure. Under the condition of ambient air inhalation hearing recovery was slowest and TTS greatest. The least TTS and fastest recovery occurred when Carbogen was inhaled. They also had subjects inhale either ambient air or thirty minutes of Carbogen following sound stimulation. When Carbogen was inhaled, recovery from TTS was more rapid than otherwise.

This repeated measure design however suffers from one flaw, air was always given first, followed by oxygen, followed by Carbogen. Therefore, order and/or practice effects can not be ruled out.

Joglekar et al. (1977) also had chinchilla inhale air, Carbogen or pure oxygen during a ten minute noise exposure. The animals that inhaled ambient air suffered greater threshold shift than either the Carbogen or oxygen groups, and Carbogen was more advantageous than oxygen. Also, chinchilla that breathed Carbogen, as opposed to ambient air, after the sound stimulation recovered from TTS faster and sustained less PTS. The authors speculated that the increased benefit of Carbogen was due to the vasodilative contribution of the carbon dioxide. One surprising result is that pure oxygen was more effective than ambient air. This is difficult to explain in view of the known effects of pure oxygen on auditory function. However, for this part of the experiment, there were only two animals per group.

Reporting a study in which auditory electrophysiologic measures were used with chinchilla, Witter et al. (1980) found that when Carbogen is inhaled prior to noise exposure, postexposure TTS is not as great as that of animals breathing compressed air. There was also less histologically observable hair cell damage in the Carbogen group. In this animal study there were only two to three ears per group. In the human

experiment by Witter et al. (1980), similar in design and result to that of Joglekar et al., except that subjects breathed either air or Carbogen before noise exposure, five subjects, tested monaurally, were used.

Harris (1979) reported a study in which chinchilla were exposed to a 133-dB SPL noise for seven minutes and the auditory brainstem electrical response threshold recovery monitored over a span of one week. Animals breathing compressed air following noise exposure retained an average threshold shift of 40 dB after one week of recovery, as compared to 20 dB for animals which breathed Carbogen after exposure. Although this is a large difference between groups, the variability within each group was also large (a 17 dB standard deviation was reported).

In the latter study there were four animals in the air group and eight in the Carbogen. Since the amount of threshold shift immediately following noise exposure was measured, it was necessary for the animals to be anesthetized during noise exposure and experimental gas inhalation, which is a shortcoming of the study. During deep anesthesia respiration is depressed (unless artificially aided). If depressed enough, carbon dioxide is retained and acidosis will occur (Lumb, 1973). Anesthetized air breathing animals may retain carbon dioxide, with resultant increase in cochlear blood flow (although with decreased oxygen supply). Acidosis may be increased even more if breathing a gas high in carbon

dioxide. Thus, anesthetized Carbogen breathing animals may become so acidic as to cause negative effect.

In an electrophysiologic and histologic experiment, Brown (1980) reported that Carbogen inhalation during acoustic exposure led to a significant protection of the cochlea from intense noise.

He used the $1\mu\text{V}$ RMS cochlear microphonic (CM) as his criterion measure in the first part of his experiment. The CM is an alternating current produced by the cochlea in response to sound. Animals were exposed to a broad band noise while breathing either Carbogen or compressed air (ten animals per group). Following noise he remeasured the amount of sound needed to obtain a $1\mu\text{V}$ CM. Although there was only a 5 dB difference between groups the results were statistically significant. "Normal auditory function" was established prior to the experiment by use of the subjective Preyer pinna reflex.

There was also statistical difference between the groups for counts of missing hair cells. Unfortunately, these counts were made using only "representative lengths" of inner and outer hair cells.

In the second experiment reported, Brown (1980) used N_1 amplitude as a criterion measure. N_1 is a negative electrical response obtained with an electrode at the round window and arises from the peripheral fibers of the auditory nerve. The

intensity of tone bursts was adjusted so as to produce N_1 responses of $50\mu V$, and then N_1 response amplitude monitored for three hours after exposure to a 4.5 kHz pure tone. There was a large variability among animals for this response and so each animal in one group was matched for sensitivity to an animal in the other group. Although variability (amount not reported) was noted, the final level of N_1 response recovery after three hours was greater in the group which received Carbogen.

Singularly, each of the Carbogen studies reviewed may be questioned in some regard, but each one has reported similar findings and taken together indicate that inhalation of Carbogen before, during, or immediately after noise exposure protects the auditory system from noise exposure. The carbon dioxide in Carbogen is no doubt a necessary ingredient since inhalation of pure oxygen is ototoxic. The importance of having the high oxygen content of 95%, found in Carbogen, as opposed to a 21% oxygen content (as found in atmospheric air) is questionable. If the protection given the cochlea by Carbogen is due to the vasodilative contribution of carbon dioxide then the excess oxygen may be of little consequence. (It should be pointed out that increased cochlear blood flow does increase the amount of oxygen available to the cochlea even when a 21% oxygen mix is inhaled.)

In the present study, noise-induced auditory brainstem electrical response (ABER) threshold shift and hair cell loss were compared in chinchilla breathing one of three possible gas mixtures: (1) air (20.94% oxygen, 0.03% carbon dioxide, 79.03% nitrogen; (2) Carbogen (95% oxygen, 5% carbon dioxide); or (3) a gas mixture of 21% oxygen, 5% carbon dioxide, 74% nitrogen. For purposes of discussion this latter gas was called Carmal (CARbon dioxide plus normal air). To avoid bias, the experimenter was not aware which animals received which gas mixture.

The hypothesis at the onset was that if carbon dioxide aids the ear in recovery from noise damage, then both Carbogen and Carmal should produce results similar to each other but dissimilar to compressed air. To test this hypothesis the following experimental questions were asked:

1. Does the auditory brainstem electrical response (ABER) threshold shift and histologic hair cell damage of animals breathing normal air following noise differ from that of animals breathing Carbogen?
2. Does the ABER threshold shift and histologic hair cell damage of animals breathing normal air following noise differ from that of animals breathing Carmal?
3. Does the ABER threshold shift and histologic hair cell damage of animals breathing Carbogen differ from that of animals breathing Carmal?

CHAPTER II

METHODS AND PROCEDURES

Fifteen standard gray chinchilla in apparent good health and between the ages of one and three years were chosen for study. Prior to experimental inclusion both ears of each animal were tested with auditory brainstem electrical response (ABER) audiometry to insure normal auditory response and provide an electrophysiologic baseline for later comparison. Normal auditory response was defined as an ABER threshold of no more than 10 dB relative to 0 dB median threshold level (MTL) for 10 ms tone bursts of 2000, 3000, and 4000 Hertz. Only one animal (which was subsequently eliminated from study) did not have thresholds within 10 dB of MTL.

Zero (0) dB MTL was established by testing 18 animals (15 experimental and 3 nonexperimental) and determining the median attenuator setting at which the thresholds were elicited for each frequency. This median attenuator setting was then called 0 MTL and all data collected were then recorded in terms decibels relative to 0 MTL.

The fifteen experimental animals were placed into three groups of five animals each, such that one group's average MTL for each frequency was no more than 2 dB from any other group's. In Tables A-1, A-2, and A-3, in the Appendix, are

found the threshold levels for the animals in terms of MTL and sound pressure level (SPL) (RMS fast and peak values given).

Instrumentation

The evoked response instrumentation is diagrammed in Figure 1. A Tychon 0133 MMD-1 processor served as an interval timer and triggering device. It interlocked a custom made switch, which turned the stimulus on and off, and initiated the scan of a signal averager, which summated the electrophysiologic response. A General Radio beat frequency generator, type 1304-B, served as tone generator; its frequency accuracy monitored with a General Radio, model 1192, electronic frequency counter.

The signal from the tone generator was gated by the custom built switch, which provided for a rise/fall time of approximately 1 ms or less. The on-time of the stimulus was 10 ms with a 90 ms off-time, identical to that used by Witter et al. (1980) and Harris (1979).

A Hewlett Packard, model 350C, attenuator set provided the necessary attenuation and the signal was transduced by a Siemens, model 25, hearing aid receiver. A probe tip, 1 cm in diameter, was attached to the receiver and placed into the animal's external auditory canal.

Electrical potentials were received by needle electrodes and amplified 5000 times by Coulbourn Instruments, model

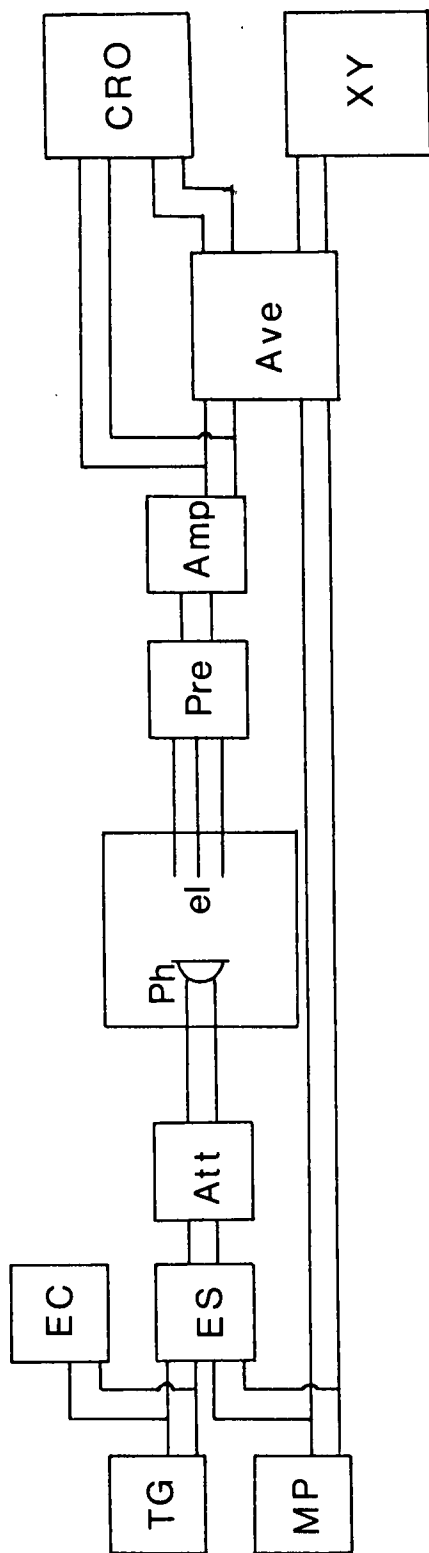


Figure 1. Block diagram of the electrical response audiometry system.

TG = tone generator

EC = electronic counter

MP = microprocessor

ES = electronic switch

Att = attenuator

Ph = earphone

el = electrodes

Pre = biological preamplifier

Amp = amplifier

Ave = summing computer (signal averager)

CRO = cathode ray tube oscilloscope

XY = X-Y plotter

575-02, differential preamplifier. Energy above 2000 Hz and below 200 Hz was filtered out by the preamplifier. The signal was then amplified again, by a factor of 10, by Coulbourn Instruments amplifier model S79-01. The amplified response was monitored on one channel of a Tektronix oscilloscope, type RM 502A, and directed to a Nicolet Instrument Corporation, model 523, averaging computer. Responses were summated by the computer and displayed on the second channel of the oscilloscope.

Calibration of the tone burst stimulus was accomplished by attaching the receiver to a Bruel & Kjaer 2 cc coupler, with associated Bruel & Kjaer inch microphone type 4144. The microphone output was directed, via a cathode follower, to a Bruel & Kjaer microphone amplifier, type 2113. Reliability of calibration procedures was assessed by measuring output levels on two separate occasions before experimentation and once after. Measured levels were always within plus or minus 1 dB.

A Nicolet digital oscilloscope, model 206, was used to determine the waveforms of the stimuli, which are shown in Figures 2, 3, and 4. The frequency spectrum of each signal was determined with a Nicolet Scientific Ubiquitous spectrum analyzer, model UA-500A. The frequency spectra are shown in Figures 5, 6, and 7.

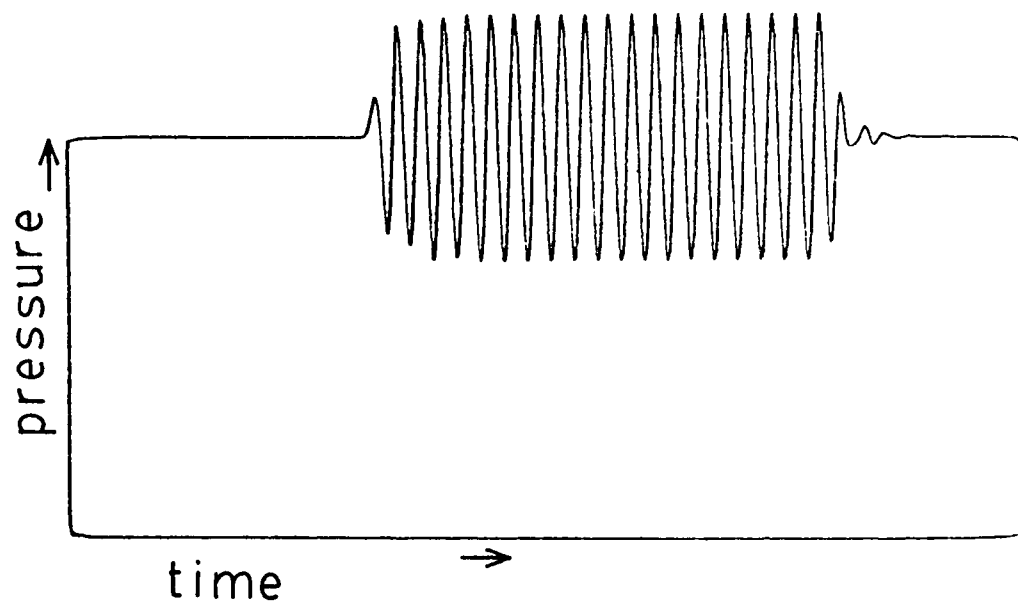


Figure 2. Acoustic waveform of the 2000 Hertz tone burst. The on-time of the tone burst is 10 milliseconds (ms).

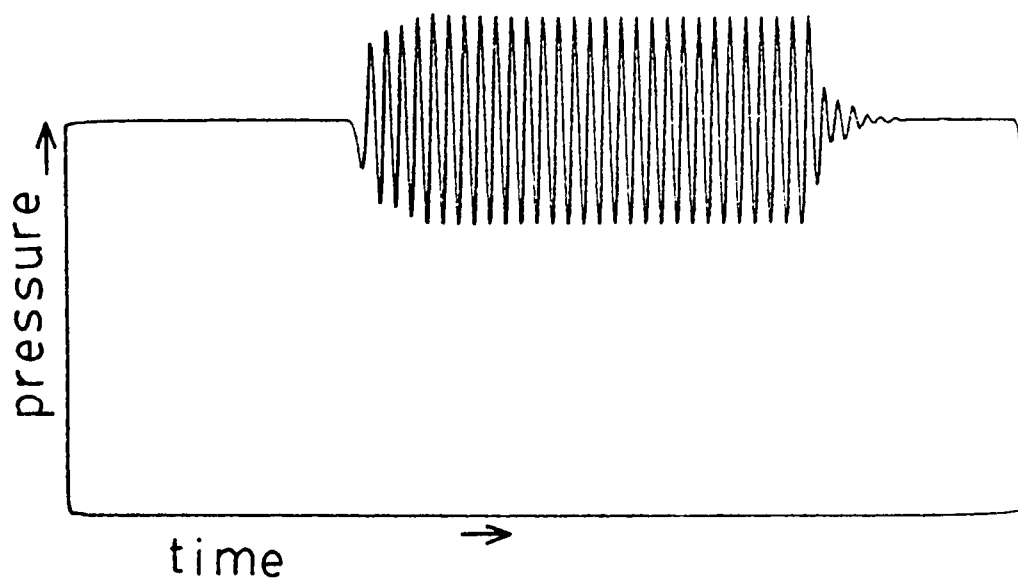


Figure 3. Acoustic waveform of the 3000 Hertz tone burst. The on-time of the tone burst is 10 milliseconds (ms).

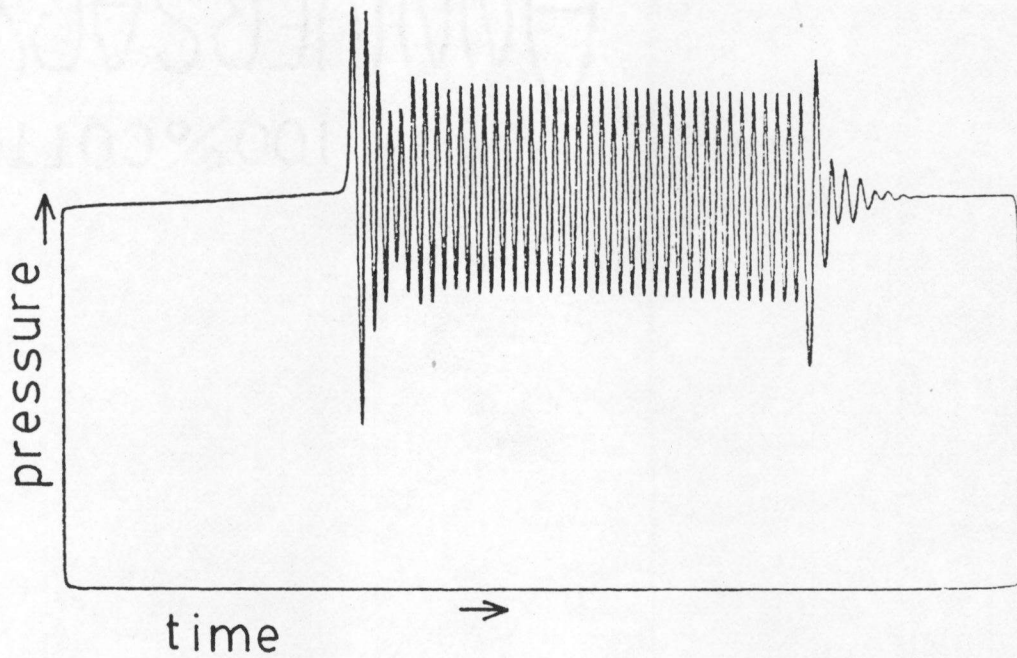


Figure 4. Acoustic waveform of the 4000 Hertz tone burst. The on-time of the tone burst is 10 milliseconds (ms).

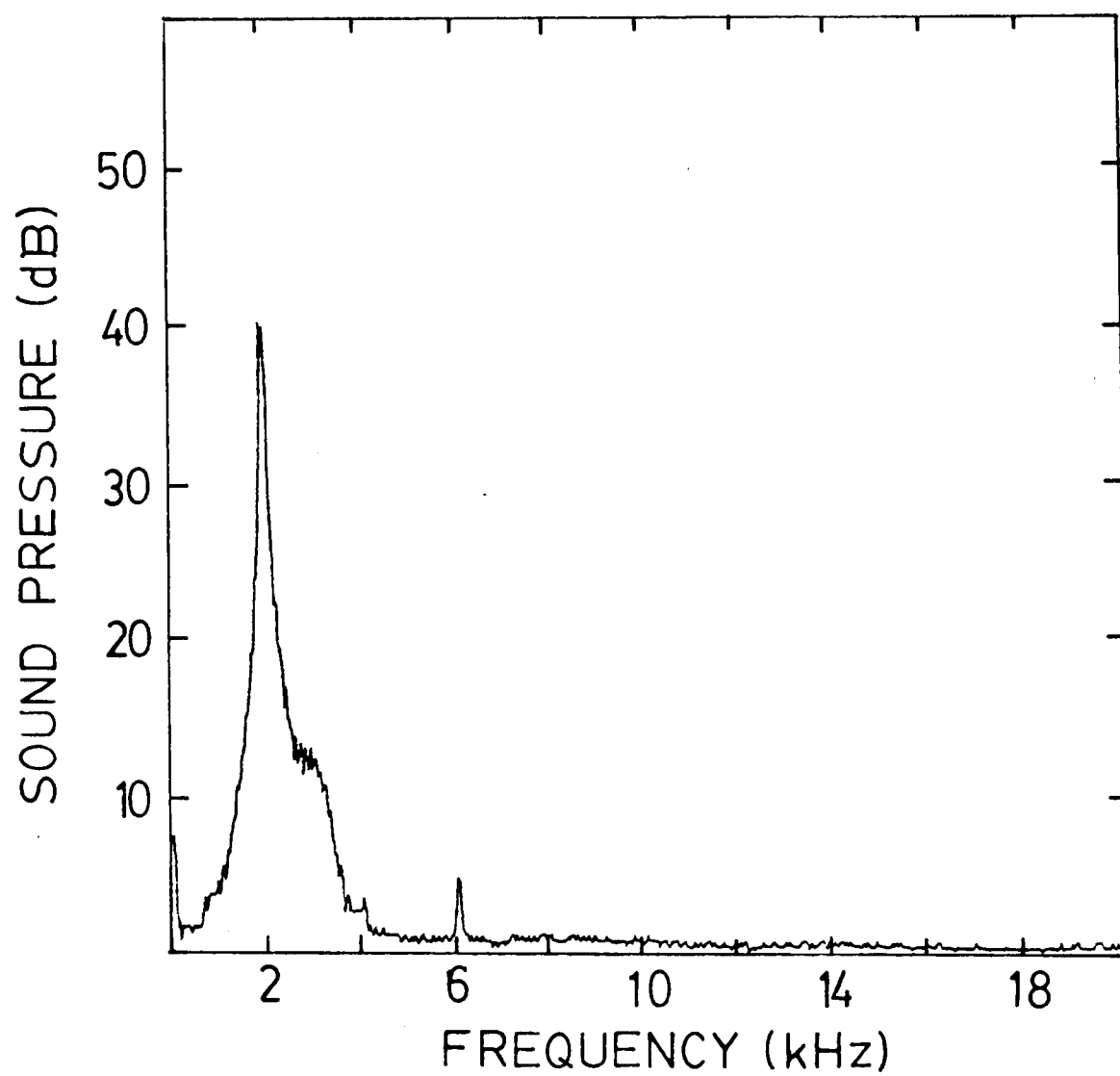


Figure 5. Frequency spectrum of the 2000 Hertz tone burst.

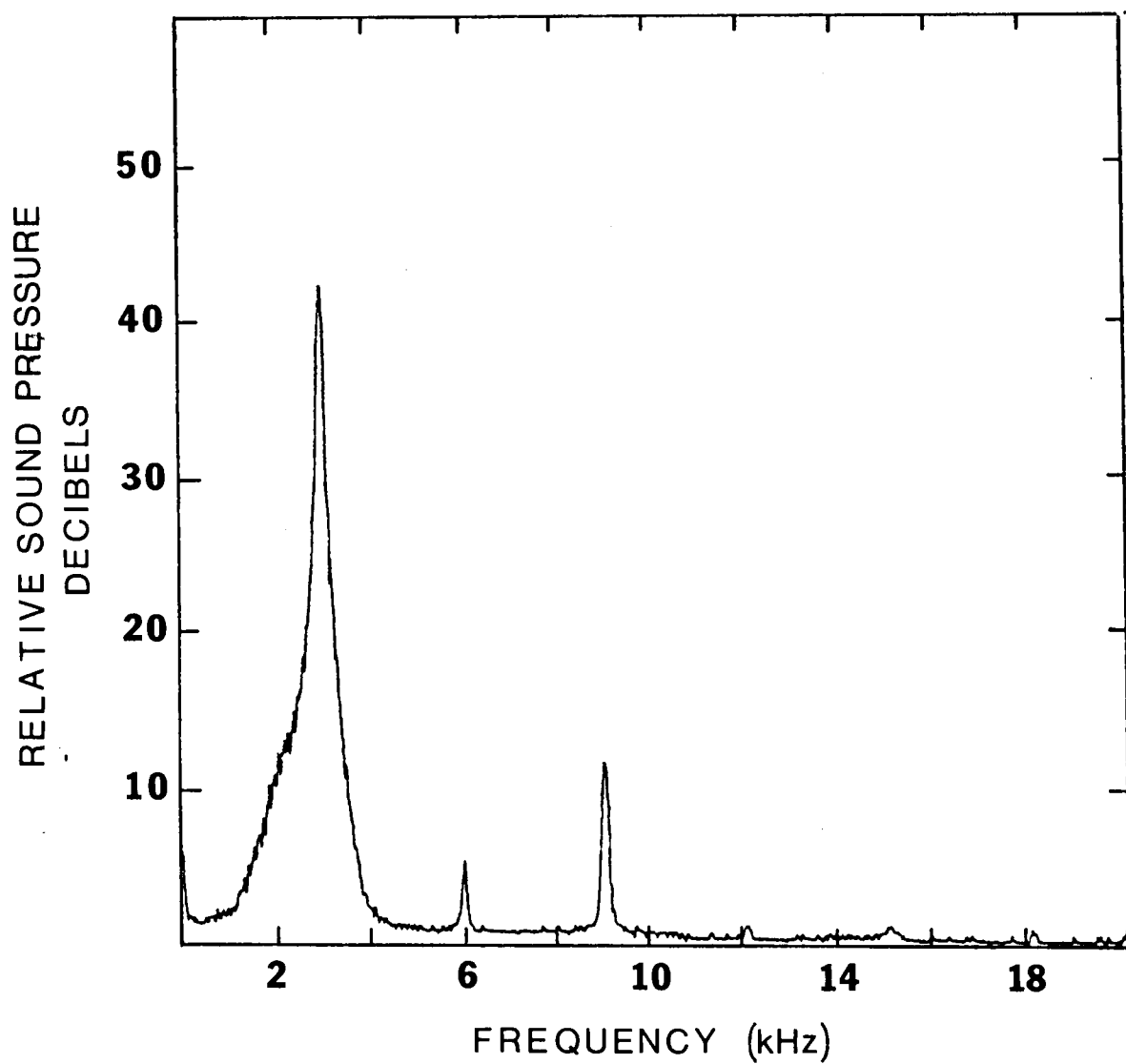


Figure 6. Frequency spectrum of the 3000 Hertz tone burst.

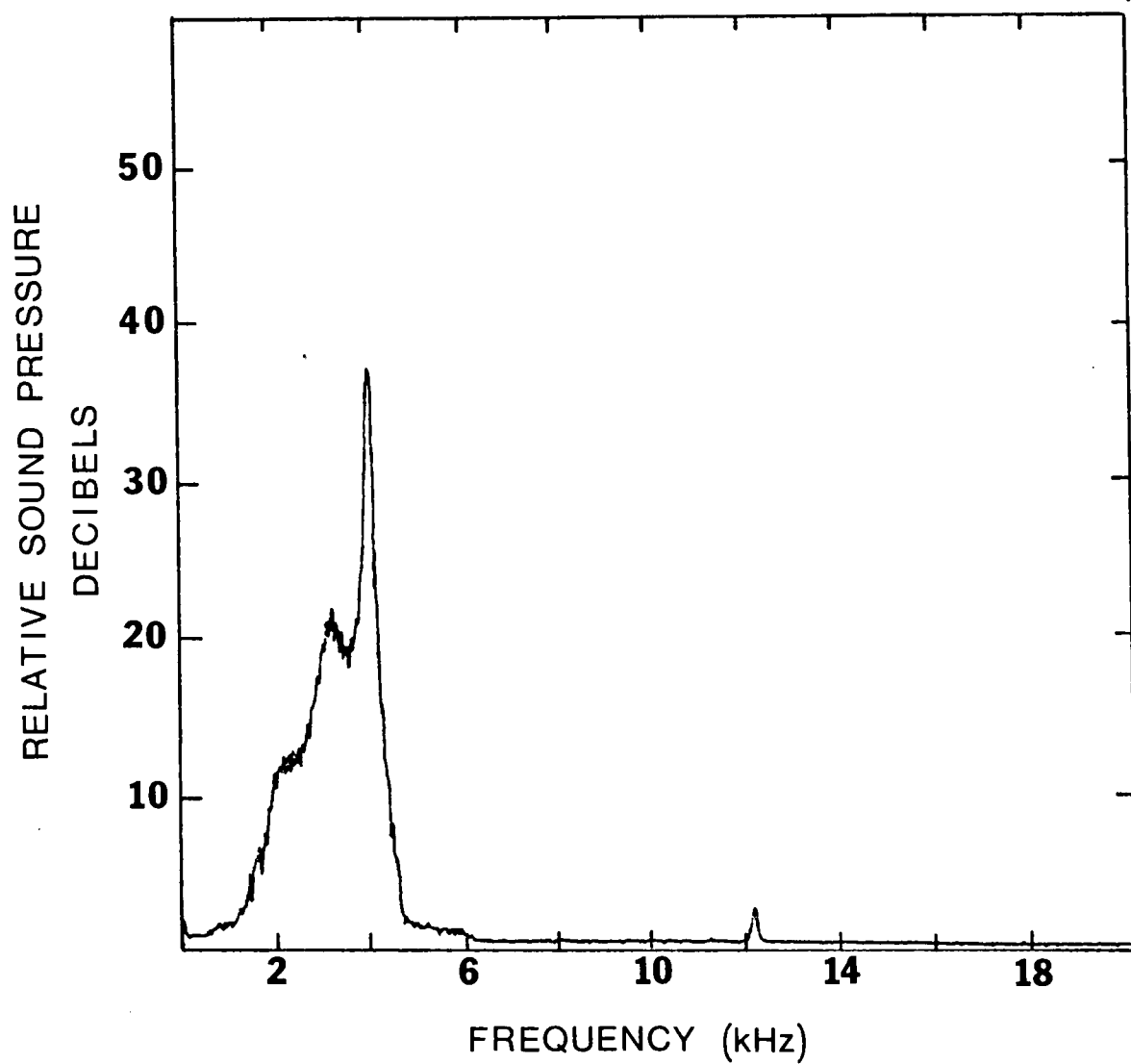


Figure 7. Frequency spectrum of the 4000 Hertz tone burst.

Anesthesia

Shortly before ABER, the animal to be tested was anesthetized with Chloropent (3.3 ml/kg body weight), injected into the peritoneal space. The peritoneal space was found by inserting the needle into the stomach region, ventral to the pelvic ridge and slightly lateral to midline. Before injection, the plunger of the syringe was withdrawn slightly to produce a bubble within the syringe and insure the peritoneal space had been located (Lipscomb, 1974).

Chloropent, formulated by Fort Dodge Laboratories, is a combination of chloral hydrate, magnesium sulfate, pentobarbital and alcohol. It was chosen over Nembutal (sodium pentobarbital) because Nembutal did not provide the depth of anesthesia necessary for ABER. It was also used instead of a combination of Ketaset (Ketamine) and Rompun (Xylazine). The Ketaset/Rompun combination provides a deep level of anesthesia but may possibly alter the obtained threshold level during times when anesthesia is deepest. It also led to respiratory arrest in 3 of approximately 15 animals (unpublished data).

While anesthetized the animal was placed on a heating pad to help maintain body heat. Adjustment of the heating pad was determined by monitoring the body temperature of the animal. Following an enema, an Electromedics series 400 temperature sensitive probe was placed, through the rectum,

4 to 4.5 cm into the animal. The probe output was read by an Electromedics, ITS 600, temperature indicator. In all cases, the temperature of the animal was maintained between 36.8 and 38.3 degrees Celsius while anesthetized. (The median temperature of 11 unanesthetized animals was 37.5.)

ABER Threshold Determination

Grass E2B subdural electrodes were placed under the skin with the first active electrode immediately over the vertex. When stimulating the left ear (which was always tested first) the second active electrode was placed approximately 1 cm caudal and 1 cm to the left of vertex. The second active was placed on the contralateral side when testing the right ear and in all instances the ground electrode was placed in the left hind paw pad.

For the determination of an ABER, 512 responses per stimulus level were summated by the computer. Summation of 512 responses is sufficient to show a threshold response and further summation does not increase the visibility of a response at the threshold level.

The second positive wave of the ABER, wave 2, was used for threshold determination. With this electrode placement and ABER procedure, wave 2 has the largest amplitude and is the most reliable wave near threshold (see Figure 8). Threshold was approached in a descending manner and defined

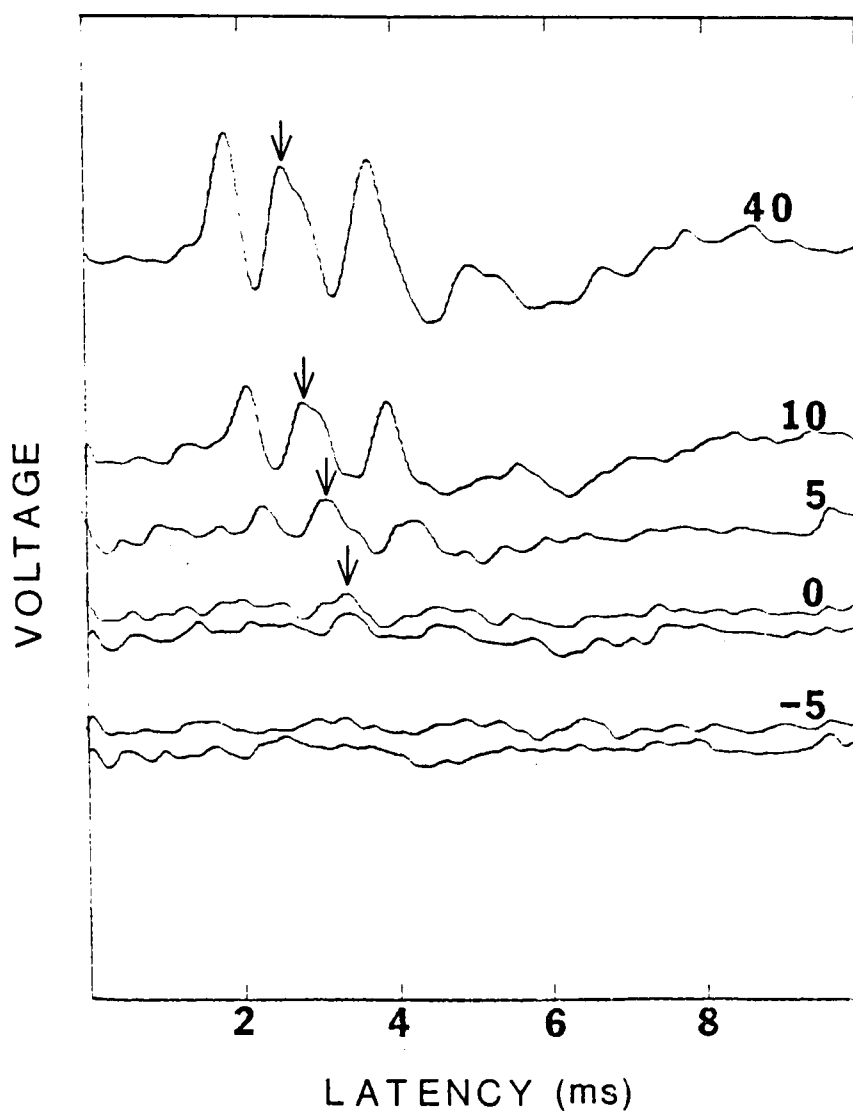


Figure 8. Sample auditory brainstem electrical response (ABER) of chinchilla. Shown are three suprathreshold responses (40, 10, and 5 dB above threshold); two threshold responses (0 dB); and two responses 5 dB below threshold. Wave 2 (indicated by arrow) was monitored for threshold determination.

as the lowest level, in 5 dB steps, which elicited an observable wave 2.

To insure that the threshold response was not spurious, two summated responses were evoked at the threshold level, stored separately in the computer, and superimposed on the oscilloscope. The responses were then visually checked for similarity and wave 2 latency. Figure 8 shows a typical ABER of chinchilla. Shown are three suprathreshold responses, two threshold responses, and two displays 5 dB below threshold. Note that as the stimulus level decreases wave 2 amplitude decreases while latency increases. Note also the similarity of the two threshold responses and identical wave 2 latency.

Reliability of ABER threshold measurement was assessed by retesting three nonexperimental animals. One animal was retested one day after the initial measurement, another animal one week after, and another 16 days after initial testing. Table 1 shows the test-retest MTLs for these three animals. In 16 of 18 threshold results, the retest threshold was either the same or 5 dB different. In only 2 of 18 instances was the retest threshold 10 dB different from the original, and in no instance was there more than a 10 dB difference.

Noise Exposure

The animals were allowed to recover from anesthesia for at least 12 days prior to noise exposure and were not

TABLE 1. Auditory brainstem electrical response thresholds for reliability determination, given in median threshold level (MTL).

	2 kHz	3 kHz	4 kHz
Animal 1*			
left	10/10	5/0	5/5
right	10/5	5/0	10/0
Animal 2			
left	0/0	0/0	0/5
right	5/0	0/5	5/5
Animal 3			
left	-5/0	0/0	0/0
right	-5/5	0/0	-5/-5

*Animal 1 was retested one day after the initial threshold determination, animal 2 one week afterwards, and animal 3, 16 days after. The slashes (/) separate the first and second test thresholds.

anesthetized during or immediately after. Respiration during anesthesia is altered and would be a difficult factor to control. Thus, animals were in their normal waking state (unanesthetized) while being exposed.

All animals were exposed to the same threshold shifting stimulus (noise); which was produced by a General Radio, model 1382, noise generator; filtered by a General Radio, model 1952, universal filter; amplified by a Heath, model AA-1640, amplifier; and transduced by a Lafayette, stock #21A 01921 sound field loudspeaker. The speaker was situated at the top of a custom built sound enclosure of approximately 3 cubic meters. The noise generator was adjusted to produce white noise and the filter set so as to produce the frequency spectrum shown in Figure 9. It is seen that the majority of the energy lies between 1000 and 2000 Hz, similar to that used by Harris (1979).

Initially, the spectrum, sound pressure, and duration of the noise were to be identical to that used by Harris (1979), except with sound field exposure instead of earphone. With available instrumentation however, 133-dB SPL exceeded equipment limits. Therefore, the intensity was decreased to 123-dB SPL and duration increased from 7 to 50 minutes. Prior to the actual experiment this amount (123-dB SPL) and duration (50 minutes) noise was found to cause extensive, but not complete, cochlear damage (hair cell loss) in two nonexperimental animals.

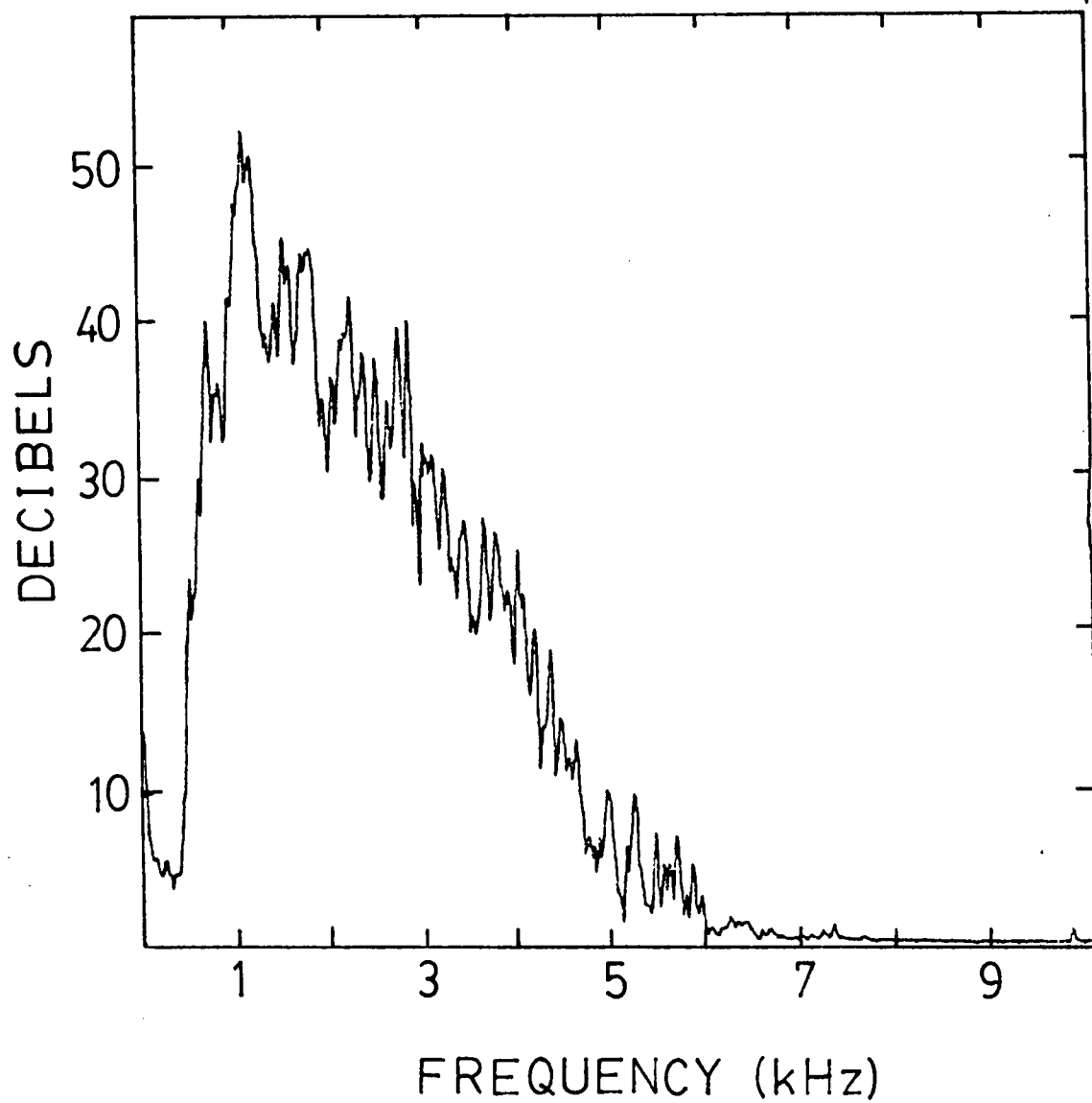


Figure 9. Frequency spectrum of the noise used to cause auditory damage.

The animal being exposed to noise was placed in a $9 \times 18 \times 10$ cm wire cage, which was placed 10 cm below the loudspeaker. After placing the animal into the wire cage, metal rods were inserted from the front to the back wall of the cage, making the effective cage smaller so that the animal could not turn around during gas inhalation. To insure that each animal received the same noise level, and to avoid the effect of standing waves, the cage was placed on a turntable which rotated at a speed of 0.33 revolutions per minute.

Each animal was exposed to the 123-dB SPL noise for 50 minutes. Calibration of the noise was accomplished by inserting a Bruel & Kjaer, model 4133, half-inch microphone through an approximately half-inch hole in the exposure booth. The microphone output led to a Bruel & Kjaer, model 2113, audio frequency spectrometer. The noise level was then checked and rechecked with the microphone in the space to be occupied by the animal's head. Three calibrations were accomplished and the measured noise level ranged from 122.5 to 123.5 dB sound pressure level (SPL).

The A-weighted sound level within the plexiglass inhalation chamber (to be discussed) was 63 dB, and within the animal holding area 50 dB.

Gas Inhalation

Following noise exposure the animal, still within the small cage, was immediately placed into a bullet shaped plexiglass breathing chamber. The appropriate gas mixture was introduced into the narrow end of the chamber at a rate of 5 liters per minute and the stream of gas directed toward the snout of the animal and out the opposite end of the chamber. The open end of the chamber was partially closed off, leaving a 1 by 5 cm opening for gas to escape.

Prior to the experiment each animal had been placed within the breathing chamber, with compressed air entering the chamber, on two separate occasions. Thus, placement of the animal within the breathing chamber during the experiment was not completely novel.

Five control animals breathed compressed air following noise exposure, five others breathed Carbogen, and five others breathed Carmal. (Carbogen is a mixture of 5% carbon dioxide/95% oxygen, and Carmal is 5% carbon dioxide/21% oxygen/74% nitrogen.) In all instances, gas inhalation began immediately after noise exposure and continued for 20 minutes. Inhalation was begun after noise exposure to be comparable to what might happen in cases of accidental noise exposure. That is, if one knows they are going to be in excessive noise they should wear hearing protection. However, if one does not know they are going to be in excessive noise, Carbogen could be made available immediately after that noise.

Fisch et al. (1976) reported that maximum perilymphatic oxygen (O_2) tension occurs in humans after 30 minutes of Carbogen inhalation, and most of the Carbogen studies have subsequently used an inhalation time of 30 minutes. Harris (1979) reported however that in chinchilla, inhalation of Carbogen for 15 minutes may be as good as or better than longer inhalation times in terms of postnoise exposure ABER thresholds. A respiration duration of 20 minutes was chosen for this study as a compromise between the 30 minute duration used by most other Carbogen studies and the 15 minute duration advocated by Harris (1979).

Carbon dioxide (CO_2) concentrations within the plexiglass breathing chamber were verified with a Beckman Medical Gas Analyzer, model LB-2. O_2 concentrations were checked with an Applied Electrochemistry Inc., model S-3A analyzer. For all gases, CO_2 amounts were within 0.5% of the specified value. Oxygen concentrations were also within 0.5% except for Carbogen, which had a measured value of 91% O_2 . The reason however was probably due to analyzer error, since it is known to become nonlinear when attempting to measure such high O_2 concentrations. Since Carbogen did have 5% CO_2 and 0% nitrogen values, it was thought that the O_2 concentration was indeed closer to 95% than the measured value indicated.

During the actual experiment the gas cannister markings were covered by a disinterested party and simply labeled

cannisters 1, 2, and 3. The experimenter was not aware which gas was administered to what group until after the experimental data were collected.

Postexposure Measures

Twenty-six days following noise exposure, ABER thresholds were again determined. Although the course of auditory recovery is of interest, thresholds were not established prior to 26 days postexposure for the following reasons:

1. Threshold determination immediately following noise exposure would necessitate that the animal be anesthetized during noise exposure and gas administration.
2. ABER threshold determination would require tone burst stimulation which, immediately following noise exposure, might alter auditory recovery.
3. Each time an animal is anesthetized there is risk of that animal not recovering from the effects.
4. Reliability of ABER measurement is not as good immediately following noise exposure because threshold is changing relatively fast over time.
5. Ultimately the most important measure of recovery from noise is permanent threshold shift, that threshold shift remaining after recovery is complete. Waiting 26 days after noise exposure should allow the auditory system to have reached asymptotic recovery.

After postexposure testing, and while still anesthetized, the animals were decapitated and the cochleae prepared for histologic study using a surface preparation technique described by Lipscomb (1974).

The inferior portion of each bulla was removed and Gluteraldehyde (5%) slowly perfused into each cochlea. The cochleae were then bathed and stored in Gluteraldehyde for no more than one week. They were postfixated with 0.5% veronal buffered osmium tetroxide solution, rinsed in distilled water, and dissected under magnification.

Next, the bone of the cochlea was drilled and chipped away, exposing the membranous labyrinth. Tectorial and Reissner's membranes were excised and one-half to one turn sections of the organ of Corti placed on a microscope slide. Using a Reichart Zeptopan microscope, equipped with interference contrast optics, a millimeter by millimeter hair cell count was done at a magnification of 1000 $\times$. A hair cell was counted as missing when there was a phalangeal scar present; or, when a number of hair cells were missing (called a wipe-out), they were counted as missing only when the basilar membrane, immediately below the wipe-out, was present.

Reliability was assessed by determining the percent total hair cell loss (right ear of animal #5) on two separate occasions. For the first count the total hair cell loss was 45.5%, for the second it was 45.7%.

CHAPTER III

RESULTS

To summarize to this point, fifteen chinchilla were exposed to a 123-dB SPL narrow band noise for 50 minutes. Immediately following noise exposure they were placed in an atmosphere of either Carbogen (5% CO₂/95% O₂), Carmal (5% CO₂/21% O₂/74% N₂), or air for 20 minutes. Each of the three groups contained five chinchilla and the average preexposure auditory brainstem electrical response (ABER) thresholds of each group were approximately equal.

Twenty-six days following noise exposure the experimenter, without knowing which gas mixture the animals received, established ABER thresholds to tone bursts, prepared the cochlea for histologic study, and conducted hair cell counts to establish the extent of noise-induced cochlear damage.

Postexposure ABER

The postexposure ABER threshold and threshold shift data are given in Appendix Tables A-4, A-5, and A-6 for animals in groups 1, 2, and 3, respectively. Group 1 contains the animals that breathed Carbogen following noise exposure, and are given the numbers 1 through 5 for identification. Group 2 contains the animals, numbered 6 through 10,

that breathed Carmal after noise exposure, and group 3 contains the animals, numbered 11 through 15, that breathed air following noise exposure.

For any given ear of an animal, the threshold shift for the three test frequencies of 2, 3, and 4 kHz tended to be similar, most thresholds being within 10 dB of any other test frequency threshold. The greatest disparity was 25 dB, occurring between 3 and 4 kHz, for the right ear of animal 12.

To facilitate group comparison, and because there was typically little difference among the 2, 3, and 4 kHz measures for a given ear, group comparisons will be made utilizing a three-frequency-average of 2, 3, and 4 kHz.

There was typically little difference between the ears of an animal in terms of threshold shift. There were two notable exceptions: the right ear three-frequency-average threshold shift of animal 4 was 22 dB and the left was 52 dB; and for animal 10, the right ear threshold shift was 35 dB and the left 20 dB. All others were within 10 dB of each other.

Referring to Table 2, the three-frequency-average threshold shift for the Carbogen animals (group 1) was 46.2 dB, for the Carmal animals (group 2) 43.5, and for the air group (group 3) 51.2 dB. Thus, the group that breathed Carmal following noise exposure suffered the least threshold

TABLE 2. Mean MTL (median threshold level), threshold shift, and standard deviation for each experimental group.

	Mean MTL	Mean Threshold Shift	Standard Deviation	Mean Threshold Shift Omitting 1 Animal per Group*
Group 1	46.5 dB	46.2 dB	10.9 dB	44.2 dB
Group 2	45.3 dB	43.5 dB	7.2 dB	40.6 dB
Group 3	48.7 dB	51.2 dB	9.7 dB	46.3 dB

*Also given is the mean threshold shift with the one animal from each group which had the greatest threshold shift. Group 1 breathed Carbogen following noise, group 2 breathed Carmal, and group 3 breathed air.

shift and the air group the most. The largest intragroup difference was 8 dB and occurred between the Carmal and air group.

As expected in the study of noise-induced auditory damage, there was considerable variability within any particular group. The standard deviation for group threshold shift ranged from approximately 7 to 11 dB (see Table 2). The least three-frequency-average threshold shift among the Carmal group animals was 20 dB, for the Carbogen group was 22 dB, and for the air group was 28 dB. The most among the Carmal animals was 57 dB, 55 dB for the Carbogen animals, and 70 dB for the air group.

At this point, it is appropriate to further consider the results of the animal which had the greatest threshold shift (70 dB) of any other animal in the study. The animal in question is #12 and belonged to the air group. Approximately ten days after noise exposure, the experimenter noted that the animal was eating infrequently and having a small number of fecal droppings. This condition continued until the postexposure testing, 26 days after noise exposure. During the postexposure testing its eyes were puffy and running fluid. Although subsequent necropsy was negative, the validity of this animal's results must be questioned because of its probable health problem following noise exposure; a health problem that could have contributed to

the large threshold shifts it suffered, which statistically appear as outliers.

For these reasons, group results were also tabulated without considering the results of animal 12 in the computations. However, since this might give the air group an advantage in terms of the mean, the results of the one animal from each group with the greatest threshold shift were also eliminated from the averaging process for the following group comparison.

The results of these computations, which eliminated the poorest scoring animal of each group, are also presented in Table 2. Under these circumstances, the mean threshold shifts were 44.2, 40.6, and 46.3 dB for the Carbogen, Carmal and air groups, respectively. As before, the Carmal group had the least threshold shift and the air group the most. The difference between the two groups is now 6 dB, as opposed to 8 dB (obtained when considering the results of all animals in the mean).

Another way to compare the three groups is to consider the absolute postexposure thresholds in MTL, instead of threshold shift. In absolute MTL the results are not different from the threshold shift results in terms of which group had the best (Carmal) and poorest (air) thresholds. In this manner however, there is less difference between the two groups: 3.4 dB.

In summary of the ABER results, group comparisons were made by considering: (1) the postexposure mean MTLs, (2) the noise induced average threshold shift considering all animals and, (3) threshold shift omitting the data from the one animal in each group which had the greatest threshold shift. The reason for this latter comparison was because the validity of the results from one animal was questionable. This particular animal had the greatest threshold shift, and so to make group comparison more equitable, the animal in each group which had the greatest threshold shift (for its group) was also omitted from computation of group means.

No matter how the results were analyzed, the Carmal group had the least postexposure MTL and threshold shift, the air group the most, and the Carbogen group in-between.

Depending on how the results were analyzed, the difference between the two most divergent groups ranged from 3.4 to 8 dB. The largest group difference was obtained when considering the threshold shift of all animals. A one-way analysis of variance of these results gave an F score of 1.1, which is not statistically significant at the 0.05 level with 2 and 27 degrees of freedom (see Table 3).

ABER/Histology Correlation

Prior to reporting the histology results, the results of correlation analysis, between ABER and histology, will be

TABLE 3. Auditory brainstem electrical response (ABER) analysis of variance table.

Source	SS	df	MS	F	p
Total	4516.96	29			
Between	340.70	2	170.3	1.1	ns
Within	4176.26	27	154.7		

The three-frequency-average threshold shift for both ears of each animal was considered.

SS: Sum of squares

df: Degrees of freedom

MS: Mean square

p: Probability

ns: Not significant (an F value of 3.35 is required for significance at the 0.05 level)

given. This should help the reader to interpret some of the histology results more clearly.

There was a positive relationship between the three-frequency-average ABER threshold shift and hair cell loss, i.e., those animals having a large threshold shift also tended to have large hair cell losses. This relationship is pictorially presented in Figure 10. The positive relationship is not perfect, as the data points are spread somewhat.

Omitting the results from two cochleae that had less than 17 mm long Corti's organs, due to dissection error, the correlation (Pearson) between ABER and total hair cell loss is 0.71.

Histology

The results of histology study are summarized in Table 4. According to the animal and ear, it shows the outer hair cell loss and total hair cell loss (inner hair cell plus outer hair cell) for each in percent. It also gives the length of each organ of Corti in millimeter (mm).

In all but two cases, dissection error was minimal (less than one millimeter error). For the right ear of animal 12 only 9 mm of Corti's organ was dissected without error—the apex was cracked during removal from the skull. For the left ear of animal 15, only 12 mm was removed without error—this time the organ of Corti was damaged or lost during drilling to thin the cochlear shell. The

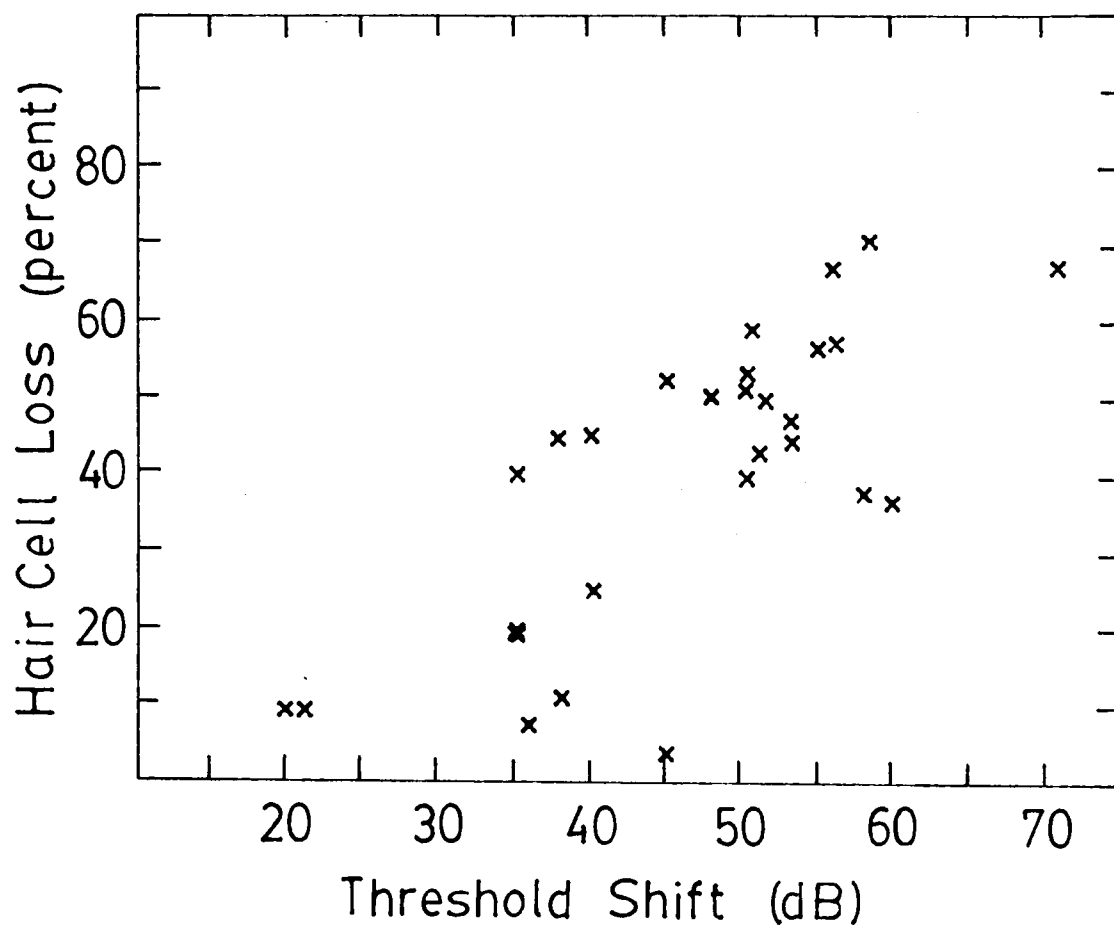


Figure 10. Relationship between the ABER three-frequency-average threshold shift and total hair cell loss. Each -x- represents the data from one ear for threshold shift and hair cell loss. Omitted from the graph are the two ears which had organs of Corti shorter than 17 mm, due to dissection error.

TABLE 4. Summary of histologic study.*

Animal/ Ear	Outer Hair Cell Loss (%)	Total Hair Cell Loss (%)	Threshold Shift (dB)	Length (millimeter)
1 R	8.9	7.8	36.7	19
1 L	13.1	11.7	38.3	18
2 R	62	52.1	50	20
2 L	56.9	47.4	53.3	20
3 R	66.8	53.9	50	19
3 L	62.4	50.3	51.7	18
4 R	10.7	9.2	21.7	19
4 L	52.8	43.5	51.7	18
5 R	54.9	45.7	53.3	18
5 L	69.1	58.6	55	19
Carbogen Group Mean	<u>45.76</u>	<u>38.05</u>	<u>46.2</u>	Median <u>19</u>
6 R	53.6	45.1	40	17
6 L	63	53.9	45	18
7 R	68.3	57.1	56.7	19
7 L	72	60.4	51.7	19
8 R	4.3	3.5	45	19
8 L	47.6	40	51.7	19
9 R	55	44.3	38.3	18
9 L	63.9	51.8	48.3	18
10 R	36	30.4	35	19
10 L	10.8	9.2	20	19
Carmal Group Mean	<u>47.45</u>	<u>39.57</u>	<u>43.5</u>	Median <u>19</u>
11 R	83	67.3	56.7	19
11 L	89	71.7	58.3	18
12 R	55.5	45.5	70	only 9 mm, due to dissec. error
12 L	83.6	67.4	71.7	17
13 R	45.3	37.6	58.3	18
13 L	42.4	36.1	60	18
14 R	30.8	25.3	40	19
14 L	25.3	20.7	35	19
15 R	24	20.4	35	17
15 L	25.4	21.8	28.3	only 12 mm, due to dissec. error
Air Group Mean	<u>50.43</u>	<u>41.38</u>	<u>51.2</u>	Median <u>18</u>

*According to the animal and ear, the percent outer hair loss, total hair cell loss, and length of each organ of Corti in millimeter is shown. For comparison, the three-frequency-average threshold shift is also reported.

portion of both cochleae which was removed without error is used in subsequent analysis, except as noted.

For all animals, the most common (mode) Corti's organ length was .19 mm. The longest was 20 mm and the shortest 17 mm (excluding the two with dissection error). The median length for groups 1 and 2 was 19 mm, and for group 3, which contains the two animals with dissection error, was 18 mm.

Inner hair cell (IHC) damage was always less than outer hair cell (OHC) damage, which can be demonstrated by comparing the OHC loss to total hair cell loss; the latter percentage always being less than the former. During dissection, differences were noted among the three rows of outer hair cells, such that in some locations one row would be damaged more or less than the other two rows. These differences were complex and their analysis is beyond the scope of this report.

As was the case with ABER, there was typically little difference between the two ears of an animal. There are notable exceptions however. For example, the right cochlea of animal 4 (group 1) had 9.2% hair cell loss and its left ear had 43.5%. This paralleled the ABER results with 29.7 and 51.7 dB threshold shift for the left and right ears, respectively.

Animal 10 (group 2) had a large difference (greater than 8% considered large) between ears with 9.2% and 30.4% hair

cell loss for the left and right ears, respectively. Again, ABER mirrored these differences.

Animal 12 (group 3) had 45.5% hair cell loss in the right and 67.4% in the left, and ABER did not positively correlate with this, the right threshold shift being 70 dB and the left 71.7 dB. The reason may be that a large portion, approximately half, of the right cochlea was damaged during dissection. The percent loss obtained from the dissection-error-free base of the cochlea may not accurately reflect the percent loss for the entire cochlea.

The most unexplainable ear difference was for animal 8 (group 2) with 40% hair cell loss and 51.7 dB threshold shift for the left ear, and 3.5% hair cell loss and 45 dB threshold shift for the right. The right ear of this animal had the least hair cell damage of any other animal in the study yet its threshold shift was more than many other animals in the study. Its preexposure thresholds, obtained one month before noise exposure, were 0 to -10 dB MTL for both ears. Although middle ear fluid was noted in none of the animals when the cochleae were being prepared for histologic study, these findings suggest that a conductive loss could have developed in the right ear following preexposure testing but before noise exposure. A conductive loss would protect, to an extent, the organ of Corti from damage but yet raise ABER thresholds. Since a conductive

loss was not verified however, this animal's results are included in all group comparisons.

Hair Cell Loss Comparison

For group comparison purposes, the results of each of the ten ears within a group were averaged. The group percentage of IHC loss, OHC loss, and total hair cell loss are given in Table 5.

The percent IHC loss was always less than OHC loss. There was usually one major area of IHC damage near the 7th to 10th mm from the base, with little IHC loss in other areas; even in areas where the OHCs were completely replaced by phalangeal scars.

The IHC loss for the animals that breathed Carbogen following noise exposure was 8.28%, for the Carmal group it was 9.34%, and for the air group it was 6.39%. Thus, the air group had the least IHC damage, while the Carmal group had the most. This difference, while small, is the only difference among the four measures of threshold shift, IHC loss, OHC loss, and total hair cell loss which indicates less auditory damage for the air group.

In terms of total hair cell loss (IHC + OHC), the Carbogen group mean loss was 38.05%, the Carmal mean loss was 39.57% and the air group mean was 41.38%. The standard deviation for each group was approximately 20%. The range for the Carbogen group was 7.8 to 58.6%, for the Carmal

TABLE 5. Percentage of IHC loss, OHC loss, and total hair cell loss for each group.

	IHC Loss (%)	OHC Loss (%)	TOTAL Loss (%)
Group 1 (Carbogen)	8.28	45.76	38.05
Group 2 (Carmal)	9.34	47.45	39.57
Group 3 (Air)	6.39	50.43	41.38

group it was 3.5 to 60.4%, and for the air group the range was 20.4 to 71.7%. While the air group did have the largest mean total hair cell loss, and did have range end points larger than either other group, there is considerable overlap among groups.

The largest histologic difference among groups is realized when comparing OHC loss. The air group had the largest OHC loss, with 50.43%, and the Carbogen group had the least with 45.76%. The Carmal group had 47.45% OHC loss. The range for the Carbogen group was 8.9 to 69.1%, for Carmal it was 4.3 to 68.3%, and for the air group the range was 24 to 89%. As was true for total hair cell loss, the air group mean and range end points were the largest but group overlap was considerable.

An analysis of variance (Bruning and Kintz, 1968) of outer hair cell loss gave an F ratio of 1.1, which is not statistically significant at the 0.05 level (with 2 and 27 degrees of freedom).

Recall that animal 12 of group 3 was thought to have been sick prior to postexposure ABER and histology preparation. Its ABER results were poorest of any other animal and the validity of including its results in group comparison was questioned. However, its histologic results were not the worst in the study, and whether or not to include them in group comparison is not a clear cut decision. By simply

removing the results of animal 12 from the air group mean, the total hair cell loss becomes 37.61% as compared to 41.38% (with #12 included); and OHC loss becomes 45.65% as compared to 50.43%.

With the results of animal 12 included, the air group has 3 to 5% greater total and OHC loss, respectively, than the Carbogen group. Without the results of #12 the difference is smaller: less than 1%.

Millimeter by Millimeter Hair Cell Damage

Since experimental gas inhalation was begun only after 50 minutes of noise had elapsed, it was expected that some permanent auditory damage might be done even before gas inhalation. To test this hypothesis, a chinchilla was placed in the same 123-dB SPL narrow band noise that was used for the main experiment. After 50 minutes of this noise it was immediately decapitated and its cochleae perfused with fixative.

The percentage of total hair cell loss for this animal is shown in Figure 11. At least for this animal, some permanent damage to the organ of Corti was present immediately following 50 minutes of the experimental noise but prior to gas inhalation. The majority of damage (11%) was located 9 mm from the cochlear base. The most damaged region within the 9th mm was a wipe-out, with inner and outer hair cells completely missing.

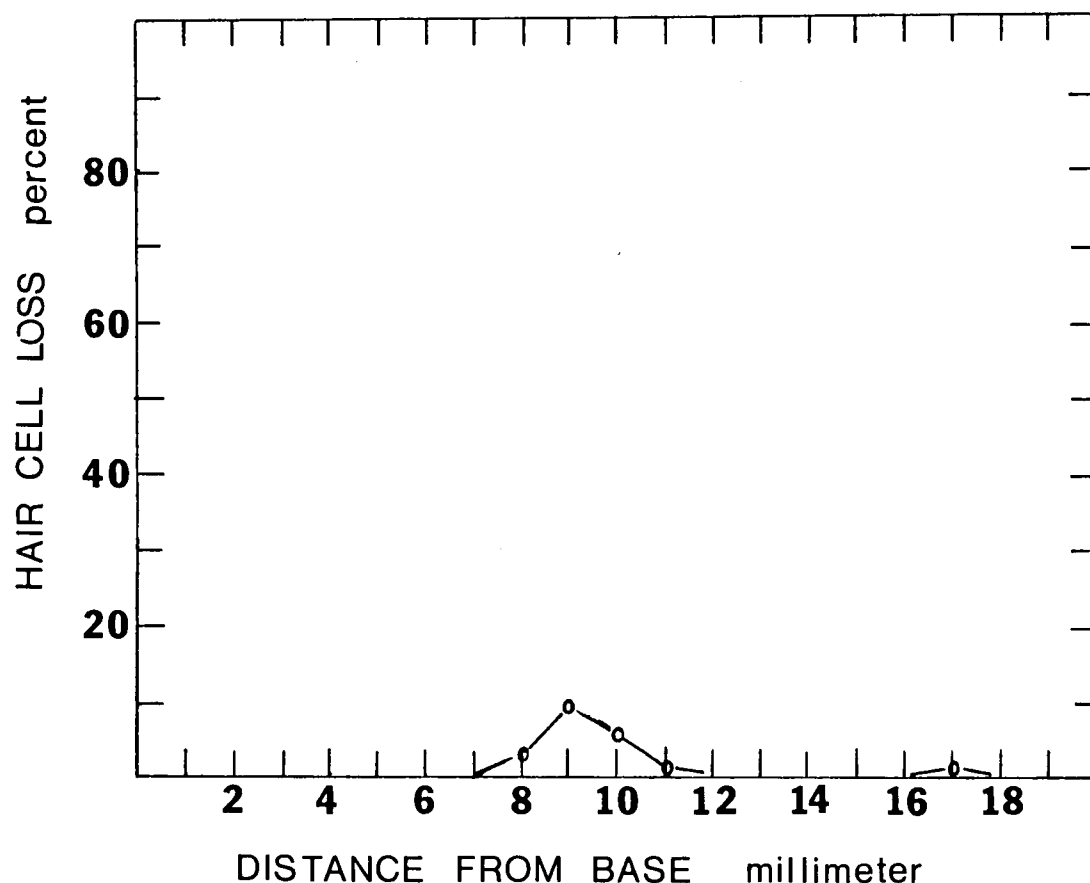


Figure 11. Percent total hair cell loss as a function of distance from the base of the organ of Corti for one nonexperimental animal. The results shown are those of a chinchilla which was placed in 123-dB SPL of noise for 50 minutes and then immediately decapitated and its cochleae perfused with fixative. The greatest sound pressure of the noise was between 1 and 2 kHz. For this animal, some permanent damage was present even immediately following noise exposure.

This is most instructive when compared to the spectrum of the noise used, which had the greatest sound pressure between 1 and 2 kHz. Eldredge et al. (1973) presented data concerning the location along the organ of Corti of maximum electrophysiologic responsitivity to a given stimulus frequency. For a 20 mm organ, they indicated that the 10th and 12th mms produce the greatest electrophysiologic output to stimuli of 1 to 2 kHz. The 9th mm corresponds to approximately 2.4 kHz (Eldredge et al., 1973). A pure tone or narrow band noise typically causes the greatest threshold shift one-half to one octave above the noise frequency. The area of damage for this one animal corresponds with these observations.

Every cochlea but the right ear of animal 8 had at least one wipe-out. The largest wipe-out was typically near the 9th to 11th millimeters, usually with the organ of Corti completely missing at the area of greatest damage, and then apical and basal to this area: supportive cells present but hair cells missing, then IHCs appearing, and then phalangeal scars appearing where OHCs should be (this latter area not considered a wipe-out). Wipe-outs with only OHCs missing but IHCs present were the most common and ranged from less than 1 mm in a few cochleae to as much as 6 mm in others. Often there were small OHC wipe-outs interspersed apical and basal to the peak damage area (near the 9th to 11th mm).

To more clearly highlight what group differences may or may not be present, a mm by mm report of total hair cell and OHC loss is given. Appendix Tables A-7, A-8, and A-9 give the histology results for each animal in terms of percent total hair cell loss per mm organ Corti. Appendix Tables A-10, A-11, and A-12 show the results in terms of OHC loss. Group means are also given.

Referring to Figure 12, which shows the average total hair cell loss for each group, the area of greatest damage for the air group was the 9th mm with 65% hair cell loss. Both other groups had greatest damage in the 10th mm, with approximately 75% total hair cell loss.

From mm 2 to mm 8, the curves derived from the results of each group differ only slightly, although the Carbogen group damage from mm 2 to 6 is largest.

From the 9th to 12th mm, the area of greatest damage for all groups, the air group consistently has less damage than either other group, with as much as 23% difference at mm 10.

There is a reversal after mm 12, with the air group consistently showing the most damage and the Carbogen group the least (this is also true for the 1st mm). The largest difference between the air and Carbogen groups, in the 13th to 18th mm region, was 20.6% at the 16th mm. In this apical part of the cochlea, the Carmal group had less damage than the air group but more than the Carbogen group. The largest

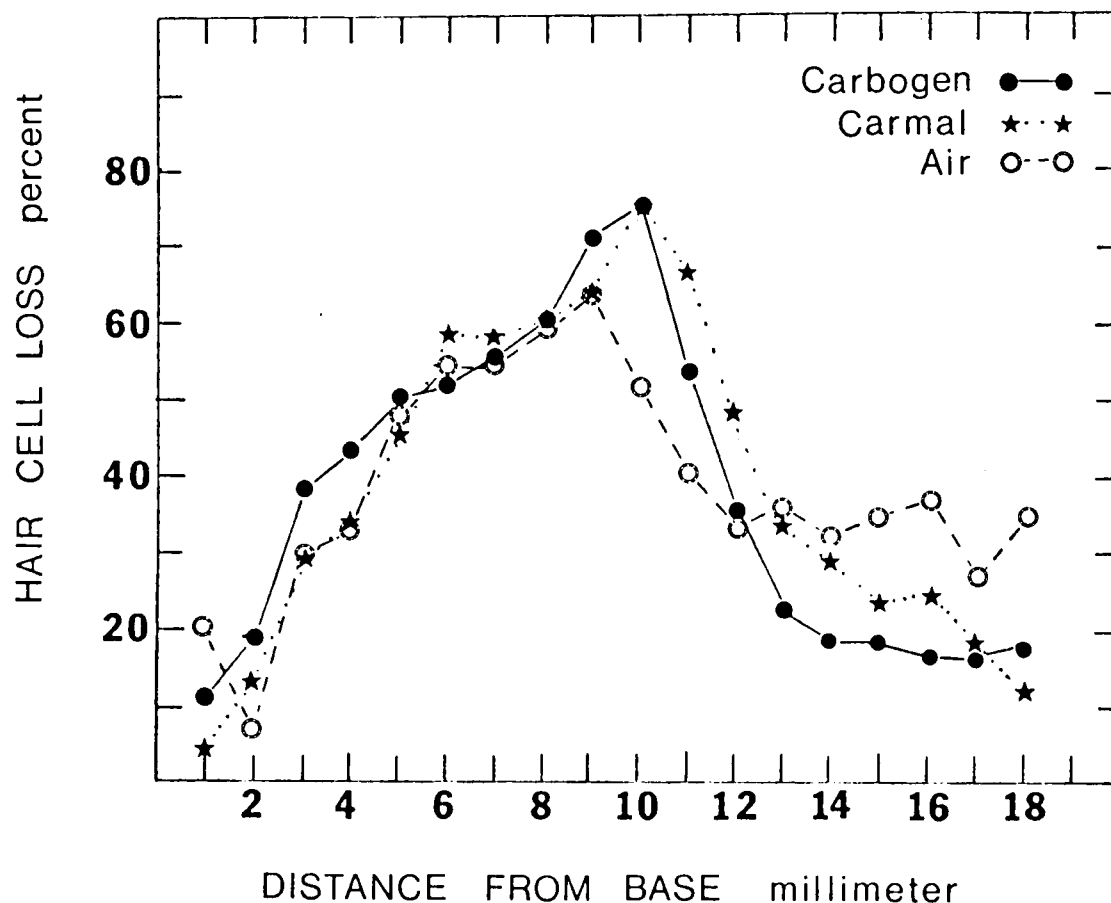


Figure 12. Percent total hair cell loss as a function of distance from the base of the organ of Corti. The results shown are the means of three groups, ten ears per group. The Carbogen group breathed Carbogen for 20 minutes immediately following 50 minutes of 123-dB SPL narrow band noise. The Carmal group breathed Carmal (5% CO₂/21% O₂/74% N₂) following noise and the air group breathed only compressed air following noise exposure.

difference between the air and Carmal groups was 21.3% in the 18th mm. The largest difference between the Carbogen and Carmal groups was 17.6% at the 12th mm, with the Carmal group having more damage.

To figuratively give an index of variability for mm by mm total hair cell loss, Figure A-1, A-2, and A-3, in the Appendix, show the standard error of the mean for the Carbogen, Carmal and air groups, respectively. There it can be seen that less variability occurred in the apical portion of the cochlea and the 1st mm.

Figure 13 displays the average OHC loss for each group. There are subtle differences between the results of OHC loss and those for total hair cell loss. For example, OHC loss shows a greater percentage of damage than total loss, and for the Carmal group the greatest OHC loss is in the 11th mm, instead of the 10th for total hair cell loss. However, the general configuration of the curves derived from OHC loss is the same for that of total hair cell loss. The groups are fairly similar at the base (excluding mm 1) with the Carbogen group showing the most damage, and the air group has the least damage from mm 7 to 11; but the most toward the apex.

Histology Summary

Those animals with large hair cell losses also tended to have large ABER threshold shifts, although there were

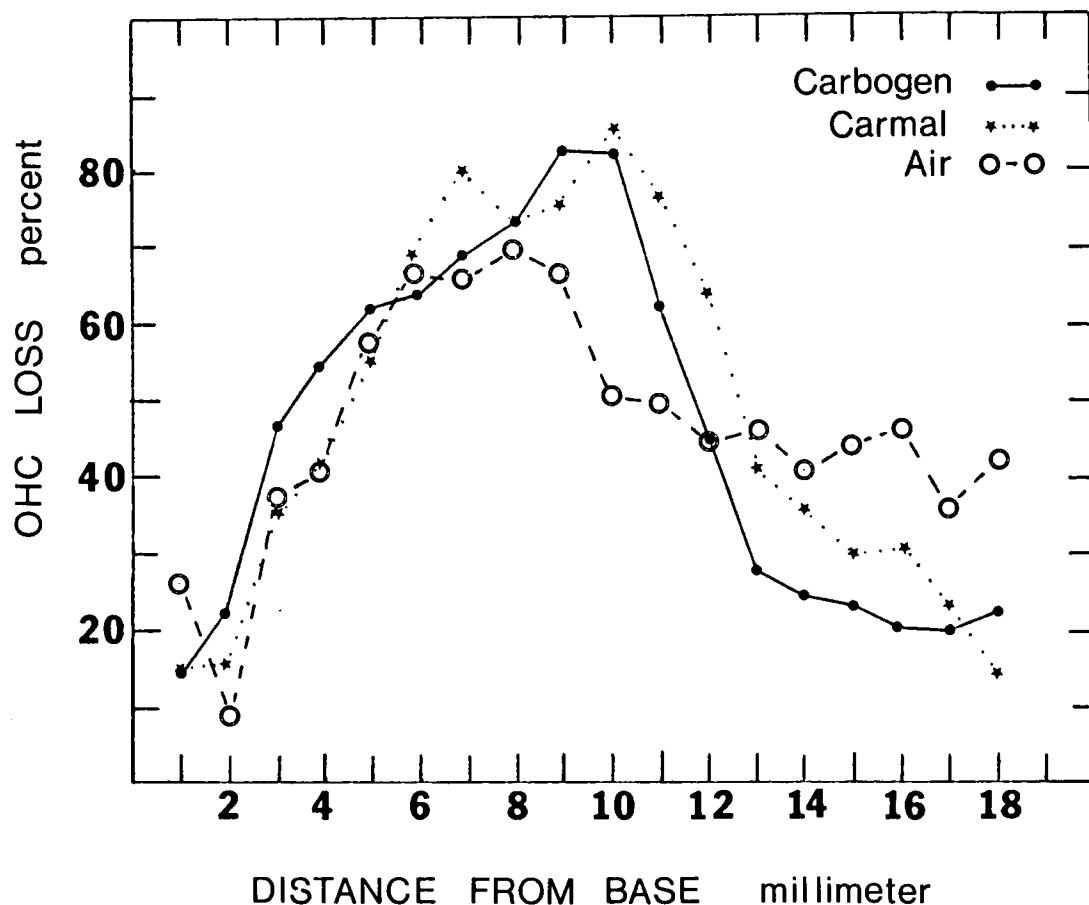


Figure 13. Percent outer hair cell loss as a function of distance from the base of the organ of Corti. The results shown are the means of three groups, ten ears per group. The Carbogen group breathed Carbogen for 20 minutes immediately following 50 minutes of 123-dB SPL narrow band noise. The Carmal group breathed Carmal (5% CO₂/21% O₂/74% N₂) following the same noise exposure, and the air group breathed only compressed air following noise exposure.

exceptions which were discussed. The correlation between the three-frequency-average ABER threshold shift and total hair cell loss was 0.71.

Measures of total and OHC loss showed that both the mean and range end points of the air group were larger (3-5% for the mean) than that for the Carbogen group, with the Carmal group in-between. However, the air group had less IHC loss than either other group.

The differences among groups were small, especially if the results of animal 12 were deleted from the air group mean, and there was considerable overlap among groups. The variability within each group was large: the standard deviation for total hair cell loss was approximately 20%. No statistically significant differences were demonstrated among groups.

A mm by mm histology group comparison was also reported. In general, the results were similar for all groups in the basal portion of the cochlea, but the air group had the least damage in the middle cochlea and the most in the apical portion.

CHAPTER IV

DISCUSSION

The search for a "magic elixer" (Ward, 1980) for noise induced auditory damage has both theoretic and practical implications. Practically, an agent which could be administered to those suffering acoustic trauma of recent onset would not only give the sufferer hope for improvement, but the actual reduction of auditory damage would allow them to lead a more normal life and reduce the need for aural rehabilitation. Theoretically, our knowledge of auditory physiology would be enhanced by knowing which agents do and do not aid auditory repair processes.

All of the Carbogen research to this point has indicated that Carbogen enhances auditory recovery following noise. Therefore it is doubtful if one could conclude from the results of this study that Carbogen does or does not reduce postnoise exposure damage. However, these results should further define the boundaries of Carbogen effects. In addition, by comparing Carmal results to those of Carbogen, the relative contribution of the high O_2 and CO_2 in Carbogen might be differentiated.

Before attempting to answer the experimental questions asked at the onset of this study, some of those factors

which help determine interpretation of the results will be discussed.

Criterion Measures

There were two basic criterion measures used in this study, one electrophysiologic and one histologic. The electrophysiologic measure used was auditory brainstem electrical response (ABER) thresholds to tone bursts. This measure was shown to be highly reliable. For reasons discussed in Chapter II, tone bursts of only three frequencies were used.

Usually the three frequencies showed approximately the same threshold shift when considering the same ear, most being within 10 dB of any other test frequency threshold. The greatest disparity was 25 dB, occurring between 3 and 4 kHz, with one other large difference of 20 dB, between 2 and 4 kHz. On the average, the 4 kHz threshold shift was 5 dB greater than the 2 kHz threshold shift, and 3 dB greater than the 3 kHz one. The three-frequency-average of these threshold shifts was used for group comparisons.

It might be expected that both ears of an animal would be equally susceptible to noise damage, since they both are part of the same body. For the most part, threshold shifts for the two ears of an animal were indeed similar (within 10 dB). There were two exceptions, with a difference of 15 dB for one animal and 30 dB for another. This raises the

question as to why these differences occurred. Some possible answers will be suggested later in this chapter.

Another question to be raised is whether one should judge damage by noise-induced threshold shift or postexposure absolute threshold. The use of threshold shift enjoys face validity because it is the amount of change brought about by noise and uses each animal as its own control. However, the absolute postexposure threshold may also be important.

Since there is a normal variation in the obtained preexposure threshold, usually reported as being ± 5 dB, threshold shift is influenced as much by the initial threshold as it is the postexposure threshold. If, due to the ± 5 dB test-retest reliability, the preexposure threshold were 5 dB high relative to the "true threshold" and then the postexposure threshold 5 dB low, the amount of threshold shift would be off by 10 dB. In other words, if an animal's true preexposure threshold was 0 dB and the postexposure threshold 40 dB, then the threshold shift would be 40 dB. However, a retest on that same animal could be within the ± 5 dB tolerance and show a preexposure threshold of 5 dB and postexposure threshold of 35 dB, resulting in a threshold shift of 30 dB. By using 10 ears per group, chances are that these occurrences would even out and lie closer than 10 dB to the "true" group threshold shift.

However, by setting a criterion of normal hearing and then grouping animals so that the average preexposure

thresholds are equal across groups, one can quantify the group absolute postexposure threshold, eliminating the ± 5 dB preexposure figure.

An infinitely long noise of, say 90 dB, would probably not produce the same threshold shift in two animals having different preexposure thresholds. It would likely shift them to the same absolute level (90 dB). If, however, the 90 dB noise ceased, the auditory system could recover to an extent. For sake of illustration, suppose that only 10 dB of recovery occurs. Comparing two animals, one with a preexposure threshold of 0 dB and the other 15 dB, the amount of threshold shift would determine the amount of change for each animal, and under these conditions would be 80 dB for one and 65 dB for the other. The absolute postexposure threshold however would best describe the auditory function of each, that is, each would be functioning at a level of 80 dB.

These explanations are oversimplified, but it should be apparent that both threshold shift and absolute postexposure threshold level are descriptively important. To relay the amount of group change and the average group level of auditory function following noise, the results presented both. There was greater difference among groups for the threshold shift data or, in other words, the groups were more similar in terms of absolute postexposure thresholds.

Histologically, the percent hair cell loss was used as a measure of auditory damage. Hair cell loss was positively correlated with ABER threshold shift. Other studies have indicated that agreement between histology and threshold measures is not always found. For example, some research has shown that almost normal thresholds can be accompanied by extensive hair cell losses (Eldredge et al., 1973; Henderson et al., 1974) while others have reported a reverse situation, with elevated thresholds and little or no hair cell loss (Hunter-Duvar and Bredberg, 1974; Dolan et al., 1975; Moody et al., 1978). Although these studies used behavioral thresholds, a positive correlation between the auditory evoked response and behavioral thresholds has been reported (Woodford, 1977).

These discrepancies may be partially explained by differences in stimulus frequencies used and the area of the cochlea used in analysis (see Lipscomb et al., 1977).

The surface preparation technique as described in 1974 and later improved by Lipscomb is, as any other technique would be, susceptible to dissection error. However, dissection error can be minimal; this study produced only two gross errors out of thirty cochleae. With more practice than that obtained by the author prior to this study, dissection error could be even less.

It is the author's opinion that, in most cases, study of the entire organ of Corti from base to apex is necessary

when analyzing hair cell damage caused by noise. This opinion is based on several observations made during this experiment. For example, in some cochleae the majority of damage would be after the 11th mm, while for others, even in the same group, it would occur before the 11th mm. Simply sampling the 11th mm would have missed the area of greatest damage in many cases. In all cochleae there would be normal areas interspersed between highly damaged ones. The use of "representative lengths" of Corti's organ (Brown, 1980) is seen as both unreliable and of questionable validity.

There were two cases for which ABER and histology were incompatible. In one case the disparity was thought due to dissection error. For the other (animal 8, right ear) not only were ABER and histology disparate, but the hair cell loss was the least of any other ear in the study. One explanation is that a conductive loss may have developed following preexposure testing but prior to noise exposure. An additional precaution which should be taken prior to study such as this is to qualify animals with tympanometry prior to noise exposure.

Negative air pressure or fluid within the Tympanum can reduce hearing sensitivity, but many times not enough to cause pretest thresholds to be greatly depressed. Thus, a middle ear problem which caused, say a 5 dB threshold shift,

would likely not cause the subject to fail preexposure testing. (Thus, a conductive loss could have been present even during the initial testing of animal 8.) However, when it comes to noise exposure, that 5 dB would make a significant difference, i.e., the cochlea would only be receiving 118 dB instead of the prescribed 123 dB (an almost four-fold reduction in energy at the cochlea).

Had the author included tympanometry in this experiment the aforementioned problem may have been avoided. On the other hand, it would have meant handling the animals more and possibly restraining or sedating them.

It was assumed, based on the literature reviewed, that if Carbogen or Carmal had an effect on auditory damage, that effect would be to metabolic factors involved in noise damage and not mechanical factors. There is evidence that some of the damage caused by the 123 dB noise was mechanical.

Recall that one animal was placed in the 123 dB noise for 50 minutes and then immediately decapitated and its cochleae perfused with fixative. As much as 11% damage at the 9th mm was present. It is probable that every experimental animal had hair cell loss prior to experimental gas inhalation.

Further evidence of mechanical damage is that every cochlea, but one, had wipe-outs. Those areas with no outer hair cells but present inner hair cells (or phalangeal scars)

ranged from less than 1 mm to as much as 6 mm. Perhaps not only did metabolic factors determine the results but mechanical factors also. This will be discussed later, in the individual susceptibility section of this chapter.

Carbogen versus Air Inhalation

Every study reviewed has indicated Carbogen to alter noise-induced auditory damage. Two had statistical inference to support that conclusion (Joglekar et al., 1977; Brown, 1980). In this study most measures showed the air group to have more damage than the group that inhaled Carbogen—but statistical significance was not reached. The differences that did occur between the two groups were small; approximately 2 to 5 dB for ABER threshold shift and 5% OHC and 2% total hair cell loss difference. Because of the large inter-group variability it must be concluded that there were no significant differences between groups for those measures.

However, while a 2% difference may not seem like a large percentage difference, in terms of actual hair cells it represents approximately 180 cells per cochlea in the chinchilla and 260 in the human. Also, the size of the differences that did occur in this study is not totally inconsistent with some of those reported in other Carbogen studies. For example:

—There was only a 5 dB difference between the Carbogen and air group results for the $1\mu\text{V}$ cochlear potential in the study by Brown,

—Brown also reported a difference between his two groups of 11% in terms of OHC loss,

—Witter et al. had only a 2% hair cell loss difference between groups in one part of their study.

The largest group difference was reported by Harris (1979) and was 20 dB; however the standard deviation in that case was approximately 17 dB. In the present study the ABER threshold shift standard deviation was 9 dB, and for histology it was 20%. In the study by Brown the standard deviation for hair cell loss was 13% for the air group animals and 5% for the Carbogen—the standard deviation for electrophysiology was not reported. In the studies by Witter et al. and Joglekar et al. with chinchilla, the group sizes were too small for statistical treatment.

Thus, the results of this study were not drastically different from the results of other Carbogen studies, except that statistical significance was reached by Joglekar et al. and Brown. These and other differences that did occur between this and other studies may, in part, be accounted for by methodological differences.

In terms of the acoustic stimulus used to cause auditory damage, it has ranged from pure tones (Joglekar et al. and

Witter et al.) to broad frequency band noise (Brown). The noise level has ranged from 100-dB SPL (Joglekar et al.) to 133-dB SPL (Harris). The type and level of noise of this study is within both of these ranges.

Perhaps the most striking difference between this study and others is the relationship between noise exposure and initiation of experimental gas inhalation. Witter et al. had subjects breathe experimental gas before noise exposure, and others (Joglekar et al. and Brown), including Witter et al., had subjects breathe experimental gas during noise exposure. Joglekar et al. and Harris did have subjects breathe the experimental gas after noise exposure, but in one case the noise exposure lasted only 10 minutes (Joglekar et al.) and in the other, experimental gas inhalation was begun after only 7 minutes of noise exposure (Harris).

In this study, experimental gas inhalation was not begun until after 50 minutes of noise, 40 minutes longer than any other study. The reason for beginning inhalation after noise exposure was to attempt to replicate what might actually occur in a nonexperimental situation. That is, after accidental noise exposure occurs to a human, Carbogen could then be administered as soon as possible following the exposure. It would be expected that the longer one waits after the noise exposure to breathe Carbogen (or Carmal), the less of an effect Carbogen or Carmal would have

on auditory repair processes. In terms of this study, a larger difference among experimental groups may have been realized had experimental gas inhalation been initiated sooner relative to noise exposure.

The most provoking result of this study is the mm by mm hair cell loss comparison. The air group had as much as 23% less damage in the middle part of the cochlea (mm 10) but 21% more in the apical part (mm 16). In these regions there is less overlap between the two groups and the results indicate that the effects of Carbogen may be more complex than previously thought, or that the physiology of the basal, middle and apical cochlea differ. Also, the results at the 1st mm were similar to those in the apex, with the air group having more damage than either other group.

One possible explanation is that, during noise exposure, there is less blood and oxygen available in the 1st mm and apical portion of the cochlea than there is in the other portions. As the cochlear artery, a terminal artery, enters the modiolus it ramifies and gives off secondary branches (Smith, 1973). In the chinchilla, the entrance of the modiolus is nearest the first cochlear turn, approximately at the 6th mm. Part of the blood supply goes basally and part goes apically, supplying the first, then second, and finally the third cochlear turn (apex). Thus, oxygenated blood gets to the parts of the cochlea distant from the

entrance of the cochlear artery only if it passes adequately from the areas closer to that entrance. If during noise exposure there is a decrease in blood supply, it might be expected that the greatest diminution in that supply would be at the portions of the cochlear vasculature most distant from the primary cochlear artery. The area most distant from the secondary branch of the cochlear artery which serves the upper turns of the cochlea is the apex. The area most distant from the secondary branch of the cochlear artery which serves the base is the 1st mm.

Of interest is that Brown reported greater histologic differences between his two groups in the apical region of the cochlea. Also, the electrophysiologic differences were greatest for the frequencies below 1500 Hz. (According to the place principle the apical portion of the cochlea is most responsive to low frequency sound pressure.) Although the noise stimulation differed between this study and that by Brown (he used broad band noise)—it is remarkable that the apical cochlea is implicated in both studies as being the area where Carbogen may reduce damage. If this holds true however, then Carbogen may have allowed significantly more damage than air in the middle cochlea.

It should also be noted that Lipscomb et al. (1977) and Vertes et al. (1979) both reported that the greatest vascular changes due to noise exposure were in the apical region of the cochlea.

The results from the middle part of Corti's organ are more difficult to explain than those of the apex and 1st mm, but again we look at blood supply for a possible explanation. Recall that the air group had less damage in the middle portion of the cochlea. If blood circulation to the apex and 1st mm is hindered during noise, then blood which ordinarily (i.e., when there is not excessive noise) circulates to those cochlear extremities may be available to the middle portions of the organ of Corti. The resultant increase (or perhaps nondecrease) of blood in that region might result in less cellular death (due to noise) in the middle cochlea. If inhalation of a gas high in CO_2 results in a more equal distribution of blood throughout the cochlea, then the middle portions of the organ of Corti may suffer more than otherwise.

Throughout this discussion it should be kept in mind that the results of any Carbogen experiment will depend in part on the noise, its spectrum and duration, and the Carbogen inhalation duration and time of initiation relative to noise termination, etc. Therefore the differences in the middle cochlea may be peculiar to this study, the apical differences occurred not only in this study but also in that of Brown.

Carmal

In view of the little or no differences between the air and Carbogen results for threshold shift and total hair cell

loss, it would be surprising if the Carmal results differed by much. For threshold shift the Carmal group differed from the air group approximately 6 dB and 3.5 dB from the Carbogen group, the Carmal animals having the least threshold shift. In the case of hair cell loss, the results for the Carmal group fell between those of the other two groups, but closest to the Carbogen group.

In the middle portion of the cochlea where the Carbogen group had more damage than the air group, the 9th, 10th and 11th mm's, the Carmal results are nearly identical to the Carbogen ones. In the 1st mm the Carmal group has less damage than the air group, and in the apex the results from the Carmal group bisect the other two, but are closer to the results of the Carbogen group, especially the more apically one looks.

The working hypothesis at the onset of this study was that CO_2 is the important constituent of Carbogen in terms of the ear's response to noise. There are no differences large enough between the Carbogen and Carmal results to discredit that hypothesis. Certainly, further research with Carmal is needed. The relative importance of the high CO_2 and O_2 in Carbogen has as many implications as that of Carbogen inhalation itself. At this point it would appear the high CO_2 concentration in Carbogen is more important than the high O_2 concentration.

Individual Susceptibility

Individual differences in susceptibility to damage from noise hampered the ability to tell if any real differences did or did not occur between groups (although large real differences would have been clearer). By real differences it is meant differences which can be generalized to noise induced auditory damage in the population.

It is common knowledge among hearing health professionals that individuals vary in their susceptibility to noise damage. Some of these differences are correlated to sex, age, personality type (stress levels) and amount of melanin in the inner ear (reviewed by Ward, 1980) but is not totally explained by these factors. The results of animal 12 of this study may give insight into this problem. It is this animal which had questionable health following noise and the greatest threshold shift. A similar result was reported by Harris in 1979; the one animal in that study with poor health also had the greatest noise induced threshold shift. I stated the hypothesis then that: "individual noise susceptibility may reflect the body's ability to maintain the cochlea under stress rather than the cochlea's innate ability to withstand stress."

A flaw in both cases is that tympanometry was not used and the presence or absence of a middle ear disorder, which could have been secondary to illness, was not determined.

It was noted earlier however that middle ear fluid was not noted in any of the ears from this study.

There were two animals which had significant postexposure differences between ears for both ABER and histology. This poses the question of why these differences occurred, since in both cases the preexposure thresholds were nearly identical for the two ears of the given animal. Also, the exposure cage within which the animals were housed during noise exposure rotated to avoid the effects of any differences in the acoustic environment within the noise exposure chamber.

This might suggest that the two ears of one animal were different in their susceptibility to damage from noise. This does not necessarily contradict the aforementioned hypothesis that noise susceptibility may be due to variability in a body's ability to furnish the cochlea with necessary nutrients and rid it of waste. The body does not always treat the two ears of an individual in exactly the same manner. It is not uncommon for disease to affect only one ear, as in hearing loss due to Mumps or Meniere's disease for example.

There is evidence that mechanical factors may play a role in individual noise susceptibility. In this study there were wipe-out areas in Corti's organ. This is consistent with mechanical damage, although mechanical and metabolic factors may be synergistic in producing wipe-outs. Wipe-out areas, with missing OHCs but present IHCs ranged from less than 1 mm

to 6 mm, and the amount of total hair cell loss was related to the amount of wipe-out, i.e., those cochleae with large wipe-outs also had large total hair cell losses. Could these ears not only have differed metabolically in their susceptibility to damage from noise, but also mechanically?

In this study, the preexposure thresholds did not predict which ears would be damaged most. In other words, those with low preexposure thresholds did not necessarily have higher or lower post-noise exposure thresholds than those with higher preexposure thresholds. However, since the test-retest reliability was approximately 5 dB, the ability to predict amount of damage from preexposure thresholds may be nonexistent. The reason is that it would not take large differences in the amount of sound energy actually transmitted to the organ of Corti to make large differences in the amount of damage. For example, an auditory system actually transmitting 123 dB to its cochlea would likely be damaged more than one with a transmission loss (say, for sake of illustration, because of middle ear impairment) of only 2 dB (actually transmitting 121 dB). This 2 dB difference however would likely not be found during preexposure testing.

The auditory sensitivity of the chinchilla is similar to that of the human (Humes and Konkle, 1980). As is true for the human, the chinchilla's external auditory canal helps

determine the amount of sound energy that reaches the organ of Corti. The external ear of the chinchilla is resonant for the frequencies between 1.5 and 8 kHz (Miller, 1970), the size of the canal being the main determinant of the resonant frequency, such that a large canal would be more resonant to lower frequencies than would a small canal. Thus, a chinchilla with an external auditory canal size such that its resonance were closest to the 1 to 2 kHz region, the most intense frequencies of the noise used in this study, would receive more energy to its inner ear than one whose canal was closer in resonance to the frequencies between 2 and 4 kHz.

To control for some factors of individual susceptibility, studies dealing with noise-induced auditory damage should control for: age, sex, pigmentation (melanin), overall health, exercise, etc.; and if feasible external auditory canal size, middle ear function, and prenoise exposure thresholds. In addition, a noise with energy spread over large range of frequencies (broad band) may be advantageous over a narrow band noise. If the frequency spectrum of a narrow band noise is close to the resonant frequency of the external canal, small differences in canal size might make large differences in the amount of sound transmitted to the middle ear and on to the inner ear. (The middle ear also determines to an extent what frequencies are transmitted most to the inner ear.)

CHAPTER V

SUMMARY AND CONCLUSIONS

Summary

Carbogen is a gaseous mixture of 95% oxygen/5% carbon dioxide, and increases cochlear blood flow. Previous experimentation has indicated it to aid auditory repair processes following noise-induced auditory damage, hypothetically as a result of improved cochlear blood and oxygen supply.

Carmal is a mixture of 21% oxygen/5% carbon dioxide/74% nitrogen. This is the first known experiment to report its effect on noise-induced auditory damage, and to compare it to Carbogen.

The purpose of this study was to determine if any differences in histologic and electrophysiologic measures occurred among three groups of chinchilla, each group breathing a different gas following noise exposure; either air, Carmal, or Carbogen.

There were five chinchilla in each of the three groups. Prior to noise exposure, auditory brainstem electrical response (ABER), a far field evoked potential occurring within the first 10 ms after stimulation, thresholds were obtained to tone bursts. The tone bursts had frequencies of 2, 3, and 4 kHz, spanning an octave above the noise,

whose major pressure was between 1 and 2 kHz, used to shift threshold. (Pure tones and narrow band noise typically produce the most threshold shift for frequencies one-half to one octave above it.) The preexposure thresholds were used to group the animals such that each group's average preexposure thresholds were within at least 2 dB of any other group's.

Following 50 minutes of noise exposure at a level of 123-dB SPL, the animal was placed, according to its group, in an atmosphere of either air, Carbogen or Carmal. The author was experimentally blind as to which kind of gas the animal was actually inhaling. Twenty-six days following this, postexposure ABER thresholds were obtained and the cochleae were prepared for histologic study using the surface preparation technique. Counts of missing hair cells at 1000 $\times$ power were made using a microscope equipped with interference contrast optics.

The three-frequency-average threshold shift for the Carmal group was 43.5 dB, for the Carbogen group was 46.2 dB, and for the air group was 51.2 dB. However, one of the animals in the air group had a probable health problem following noise and the validity of its results questionable. Its ABER threshold shift was the largest in the study. For this reason, another ABER group comparison was made which eliminated the poorest scoring animal from each group. In

this manner, in order of increasing threshold shift, the Carmal group had 40.6 dB threshold shift, the Carbogen group 44.2, and the air group 46.3 dB. In addition to the air group mean being the largest, its range end points were also the largest. However, group overlap was also large and no statistically significant differences were demonstrated.

The percent IHC loss was relatively small with 6.39% IHC loss for the air group, 8.28% for Carbogen and 9.34% for Carmal, the air group having the least IHC damage.

The largest histologic difference, in terms of a single total, was obtained by comparing complete OHC loss. In order of increasing damage, there was 45.75% OHC loss for the Carbogen group, 47.45% for Carmal, and 50.43% for the air group. As was true for ABER, the air group had the largest mean and range end points but the differences among groups did not reach statistical significance.

The largest group differences were realized when comparing hair cell loss per distance from the base of the cochlea, in mm. The air group had less damage than either other group in the mid portion of the cochlea, the area of greatest damage for all groups, but the most damage in the apical portion and 1st mm. A previous study (Brown, 1980) has also implicated the apical cochlea as the area where Carbogen may reduce damage. Carmal was not found to differ significantly from Carbogen.

Conclusions

With the results of this study in mind, as well as results from other Carbogen studies, the following conclusions are indicated:

1. Whether chinchilla breathed air or a gas mixture high in carbon dioxide, differences in auditory damage following postnoise exposure experimental gas inhalation did not reach statistical significance. Other Carbogen studies however have reported statistically significant differences between Carbogen and air inhalation. The reason this study did not may be because experimental gas inhalation did not begin until after 50 minutes of noise exposure (longer than any other Carbogen study).

There was, however, a tendency for the animals breathing Carbogen or Carmal to differ histologically from the air breathing group, depending on the area of the organ of Corti in question. A high carbon dioxide mixture may indeed protect the 1st mm and apex of Corti's organ, but possibly at the expense of the midportion of the organ.

2. Noise induced auditory damage does not differ if chinchilla breathe Carbogen or Carmal following noise. There is no known previous research in this regard and experimental procedures different from this study's may produce a different conclusion. However, it must be concluded at this point that carbon dioxide is the important constituent of Carbogen in

terms of its effect on auditory damage. Increased blood flow as a result of carbon dioxide is more important than hyper-oxygenation as a result of high oxygen concentration.

3. Individual differences in susceptibility to noise-induced auditory damage is large and may mask real differences between experimental treatments.

4. Differences in individual susceptibility may in part be explained by the overall health status of the individual in question.

Implications for Further Study

Based on the literature reviewed and experience gained during this research project, the following suggestions are made.

1. Further study of Carbogen and Carmal inhalation for noise-induced auditory damage is recommended, using procedures dissimilar to those used in this report. Although it is the author's opinion that enough is known about Carbogen inhalation for its practical application to cases of recent acoustic trauma, there is still a paucity of information on its exact mechanism of protection and the important parameters involved. To maximize differences between air, Carbogen, and Carmal breathing groups, noise exposure and gas inhalation should be simultaneous. The longer one waits after noise exposure to breathe Carbogen or Carmal, the less of a chance they have of altering auditory repair processes. Once the

function of CO_2 and O_2 in noise damage is known and understood, then study of postexposure inhalation can tell us something more about the practicality of Carbogen (or Carmal) inhalation; that is, how long after noise exposure can Carbogen be inhaled and still be of benefit.

2. Also recommended is study of noise-induced auditory damage following administration of cerebral vasodilators other than carbon dioxide.

3. Although the point to be made was discussed in Chapter I, it is reemphasized: in studies such as this one, the animal should not be anesthetized during noise exposure and experimental gas inhalation. Anesthesia can result in reduced respiration and increased blood acidity. Air-treated animals may derive some of the recuperative benefits, if there are any, that accompany a slight acidosis. A combination of acidosis secondary to anesthesia in addition to that caused by CO_2 inhalation may cause so great a pH change that negative effects are caused.

4. Controversy still exists concerning the role of cochlear blood flow in the pathogenesis of noise-induced auditory damage. A helpful study would be one in which cochlear blood flow is actually measured before, during and after acoustic insult and then blood flow change correlated with acoustic damage.

5. In studies using noise as a stressor but in which something other than the noise itself is the main area of

interest (e.g. Carbogen), that noise should be flat and broad band. Because of differences among individuals in the frequencies of maximum transmission to the inner ear, a pure tone or narrow band noise might be expected to cause a more different stapedial displacement from ear to ear than would a broad band, flat, noise.

6. Further research is needed concerning individual susceptibility differences to damage from noise. One thing which needs to be addressed is the interaction of external auditory canal size, frequency of stimulation, and amount of noise damage. [As an aside, hyperinsulinism and hyperlipidemia may also be related to individual noise susceptibility (see Spencer, 1980)].

7. In all studies comparing groups for noise-induced auditory damage, a hearing test and tympanometry should be completed prior to noise exposure. The reasons were discussed in Chapter IV.

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APPENDIX

TABLE A-1. Prenoise exposure threshold levels in median threshold level (MTL), RMS fast, and peak hold for the animals in group 1, the Carbogen group.*

Animal/ Ear	Frequency (kilohertz)	Median Threshold Level (dB)	Sound Pressure Level	
			RMS Fast (dB)	Peak Hold (dB)
1L	2	0	12.5	26
	3	-5	5	8
	4	-5	-10	7
1R	2	0	12.5	26
	3	0	10	13
	4	-5	-10	7
2L	2	0	12.5	26
	3	0	10	13
	4	-5	-10	7
2R	2	5	17.5	31
	3	-5	5	8
	4	0	-5	12
3L	2	10	22.5	36
	3	5	15	18
	4	0	-5	12
3R	2	5	17.5	31
	3	5	15	18
	4	0	-5	12
4L	2	5	17.5	31
	3	0	10	13
	4	0	-5	12
4R	2	5	17.5	31
	3	0	10	13
	4	0	-5	12
5L	2	0	12.5	26
	3	-5	5	8
	4	0	-5	12
5R	2	0	12.5	26
	3	0	10	13
	4	0	-5	12

*The mean MTL is 3, -0.5, and -0.5 for 2, 3, and 4 kHz, respectively.

TABLE A-2. Prenoise exposure threshold levels in median threshold level (MTL), RMS fast, and peak hold for the animals in group 2, the Carmal group.*

Animal/ Ear	Frequency (kilohertz)	Median Threshold Level (dB)	Sound Pressure Level	
			RMS Fast (dB)	Peak Hold (dB)
6L	2	5	17.5	31
	3	5	15	18
	4	5	0	17
6R	2	5	17.5	31
	3	5	15	18
	4	5	0	17
7L	2	5	17.5	31
	3	10	20	23
	4	5	0	17
7R	2	5	17.5	31
	3	10	20	23
	4	5	0	17
8L	2	0	12.5	26
	3	-10	0	3
	4	-10	-15	2
8R	2	0	12.5	26
	3	-10	0	3
	4	-10	-15	2
9L	2	-5	7.5	21
	3	-10	0	3
	4	-5	-10	7
9R	2	0	12.5	26
	3	-5	5	8
	4	0	-5	12
10L	2	10	22.5	36
	3	5	15	18
	4	5	0	17
10R	2	10	22.5	36
	3	5	15	18
	4	5	0	17

*The mean MTL is 3.5, -0.5, and 0.5 dB, for 2, 3, and 4 kHz, respectively.

TABLE A-3. Prenoise exposure threshold levels in median threshold level (MTL), RMS fast, and peak hold for the animals in group 3, the air group.*

Animal/ Ear	Frequency (kilohertz)	Median Threshold Level (dB)	Sound Pressure Level	
			RMS Fast (dB)	Peak Hold (dB)
11L	2	0	12.5	26
	3	-5	5	8
	4	-5	-10	7
11R	2	0	12.5	26
	3	0	10	13
	4	0	-5	12
12L	2	5	17.5	31
	3	0	10	13
	4	-5	-10	7
12R	2	10	22.5	36
	3	0	10	13
	4	-5	-10	7
13L	2	-10	2.5	16
	3	0	10	13
	4	5	0	17
13R	2	-5	7.5	21
	3	0	10	13
	4	0	-5	12
14L	2	5	17.5	31
	3	0	10	13
	4	-5	-10	7
14R	2	0	12.5	26
	3	0	10	13
	4	-5	-10	7
15L	2	10	22.5	36
	3	0	10	13
	4	5	0	17
15R	2	5	17.5	31
	3	0	10	13
	4	0	-5	12

*The mean MTL is 2, -1.5, and -1.5 dB for 2, 3, and 4 kHz, respectively.

TABLE A-4. Threshold levels and threshold shifts for the animals that inhaled Carbogen (group 1) following noise exposure.

Animal	Ear	Median Threshold Level (dB)				Threshold Shift (dB)			
		2*	3	4		2*	3	4	
1	L	35	35	35		35	40	40	
1	R	35	35	35		35	35	40	
2	L	55	55	45		55	55	50	
2	R	50	45	55		45	50	55	
3	L	55	55	60		45	50	60	
3	R	55	50	55		50	45	55	
4	L	55	50	55		50	50	55	
4	R	25	20	25		20	20	25	
5	L	50	55	55		50	60	55	
5	R	50	55	55		50	55	55	
Mean		46.5	45.5	47.5		43.5	46	49	

*Frequency kilohertz.

TABLE A-5. Threshold levels and threshold shifts for the animals that inhaled Carmal (group 2) following noise exposure.

Animal	Ear	Median Threshold Level (dB)				Threshold Shift (dB)			
		2*	3	4		2*	3	4	
6	L	50	50	50		45	45	45	45
6	R	45	45	45		40	40	40	40
7	L	60	60	65		50	55	55	55
7	R	65	65	65		55	60	55	55
8	L	50	45	45		50	55	55	55
8	R	35	40	40		35	50	50	50
9	L	40	40	45		45	50	50	50
9	R	35	40	35		35	45	35	35
10	L	30	25	25		20	20	20	20
10	R	45	40	40		35	35	35	35
Mean		45.5	45	45.5		41	45.5	44	44

*Frequency kilohertz.

TABLE A-6. Threshold levels and threshold shifts for the animals that inhaled compressed air (group 3) following noise exposure.

Animal	Ear	Median Threshold Level (dB)				Threshold Shift (dB)			
		2*	3	4		2*	3	4	
11	L	55	55	50		55	60	55	
11	R	55	60	55		55	60	55	
12**	L	65	65	75		70	65	80	
12**	R	65	55	75		75	55	80	
13	L	50	55	55		60	55	65	
13	R	50	55	55		55	60	60	
14	L	35	35	35		30	35	40	
14	R	35	40	40		35	40	45	
15	L	30	25	35		20	25	40	
15	R	35	30	35		40	30	35	
Mean		47.5	47.5	51		49.5	48.5	55.5	

*Frequency kilohertz.

**Animal #12 became sick approximately one week after noise exposure.

TABLE A-9. Percent of total hair loss for the animals in group 3 (air).

Distance from base (mm)	Animal/Ear														Mean
	11/R	11/L	12/R	12/L	13/R	13/L	14/R	14/L	15/R	15/L					
1	33.1	29.0	28.4	52.8	1.7	53.3	0	1.8	1.1	4.2					20.54
2	31.9	0	0	29.6	4.6	6.3	0.2	0.4	0.2	0.2					7.34
3	77.3	78.2	61.3	62.0	1.3	0.6	0.2	1.1	0.4	26.1					30.85
4	88.0	78.4	71.2	80.3	5.7	3.4	0	0.2	4.2	4.2					33.56
5	75.4	78.2	79.6	80.0	49.6	28.8	40.9	0.4	15.0	25.0					47.30
6	76.1	75.0	79.8	77.5	76.1	65.1	0.2	0.6	34.9	59.5					54.48
7	75.8	75.6	73.1	71.6	76.9	79.4	0	1.3	34.9	54.8					54.34
8	78.6	78.4	73.5	79.4	81.1	98.3	0.2	1.9	91.2	8.8					59.14
9	77.9	77.1	79	74.1	84.7	86.6	1.0	18.3	91.2	59.5					64.94
10	78.2	85.9	79	----	92.4	70.0	5.9	56.7	2.5	3.8					52.71
11	80.3	80.5	38.2	----	42.6	30.3	77.3	8.8	1.7	8.8					40.95
12	81.1	79.8	27.3	----	25.6	16.2	28.4	36.3	3.2	6.9					33.86
13	81.1	77.5	24.6	----	25.6	19.3	12.2	58.8	2.3	----					37.67
14	78.6	64.9	20.8	----	29.8	22.3	19.7	25.0	0.8	----					32.74
15	68.1	63.9	12.4	----	30.9	20.2	66.8	21.2	1.5	----					35.63
16	61.3	59.5	16.6	----	23.1	9.0	57.6	38.4	39.5	----					38.12
17	58.8	72.1	8.2	----	8.2	3.2	45.0	30.3	4.2	----					28.74
18	0	58.5	----	----	2.1	51.5	62.4	46.6	----	----					36.85
19	44.2	----	----	----	----	----	----	43.5	----	----					50.15
20	----	----	----	----	----	----	----	----	----	----					----

TABLE A-10. Percent of outer hair cell loss for the animals in group 1 (Carbogen).

[illegible]

TABLE A-12. Percent of outer hair cell loss for the animals in group 3 (air).

Distance from base (mm)	Animal/Ear										Mean
	11/R	11/L	12/R	12/L	13/R	13/L	14/R	14/L	15/R	15/L	
1	42.4	36.2	35.8	67.7	1.9	68.5	0	2.3	0.9	5.0	26.07
2	39.7	0.0	0.0	34.4	5.3	7.4	0	0.5	0.3	0.3	8.79
3	92.6	97.4	75.7	76.2	1.3	0.5	0	1.3	0.5	32.8	37.83
4	97.6	97.4	87.8	100	6.6	4.0	0	0	5.2	5.3	40.39
5	93.4	97.9	98.7	99.7	60.6	35.4	43.9	0.5	20.0	31.5	58.16
6	92.6	93.9	100	96.0	93.9	81.7	0	0.8	43.9	74.9	67.77
7	94.2	94.4	91.3	87.8	96.6	99.5	0	1.3	43.9	60.8	66.98
8	97.4	98.4	90.5	98.7	100	100	0	2.4	100	11.1	69.85
9	96.6	97.1	0.0	92.6	100	97.9	0.8	21.7	100	60.8	66.68
10	97.9	97.6	0.0	----	98.7	79.6	7.41	65.9	3.2	4.8	50.57
11	100	100	45.5	----	53.7	37.8	88.1	8.7	1.9	9.3	49.41
12	100	100	33.6	----	30.7	19.8	35.2	42.6	4.0	8.7	44.96
13	100	97.1	30.7	----	31.2	23.5	15.3	72.5	2.9	----	46.65
14	97.6	81.5	25.9	----	37.0	27.8	24.9	31.5	1.1	----	40.91
15	84.7	78.3	15.6	----	38.1	25.4	82.8	26.2	1.9	----	44.12
16	75.7	73.5	19.8	----	28.8	11.4	72.9	48.4	42.3	----	46.6
17	73.5	90.7	10.1	----	10.3	4.0	56.3	38.1	5.3	----	36.04
18	0.0	73.7	----	----	2.6	38.9	78.6	58.7	----	----	42.08
19	52.9	----	----	----	----	----	79.0	54.8	----	----	----
20	----	----	----	----	----	----	----	----	----	----	----

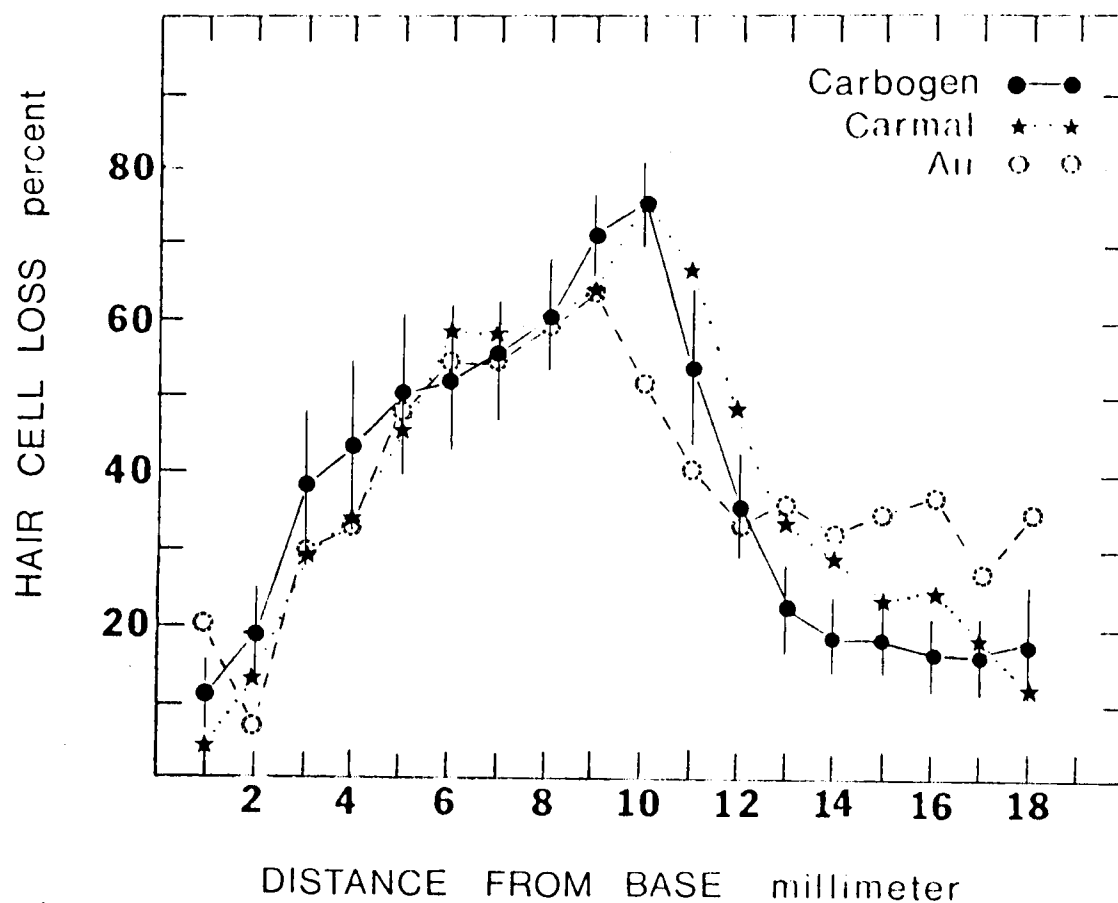


Figure A-1. Average percent total hair cell loss showing the standard error of the mean (vertical lines) for the Carbogen group.

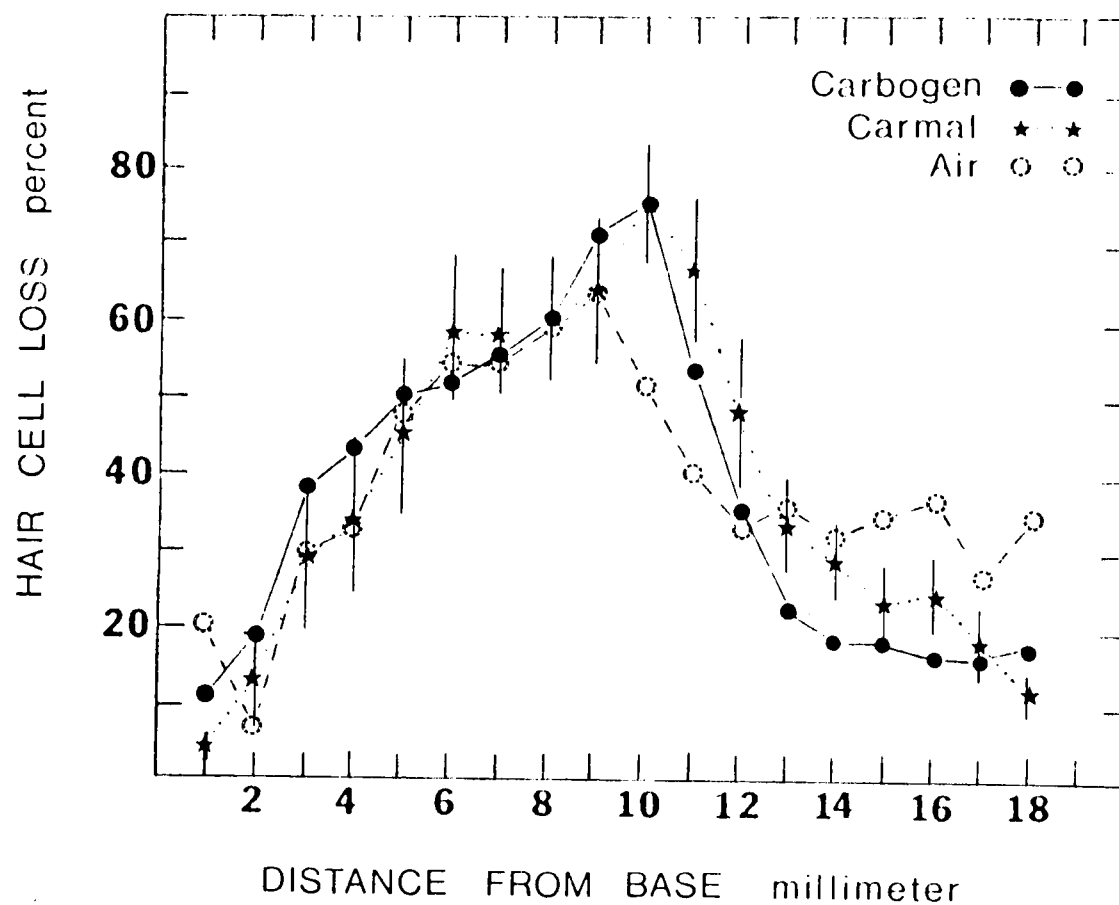


Figure A-2. Average percent total hair cell loss showing the standard error of the mean (vertical lines) for the Carmal group.

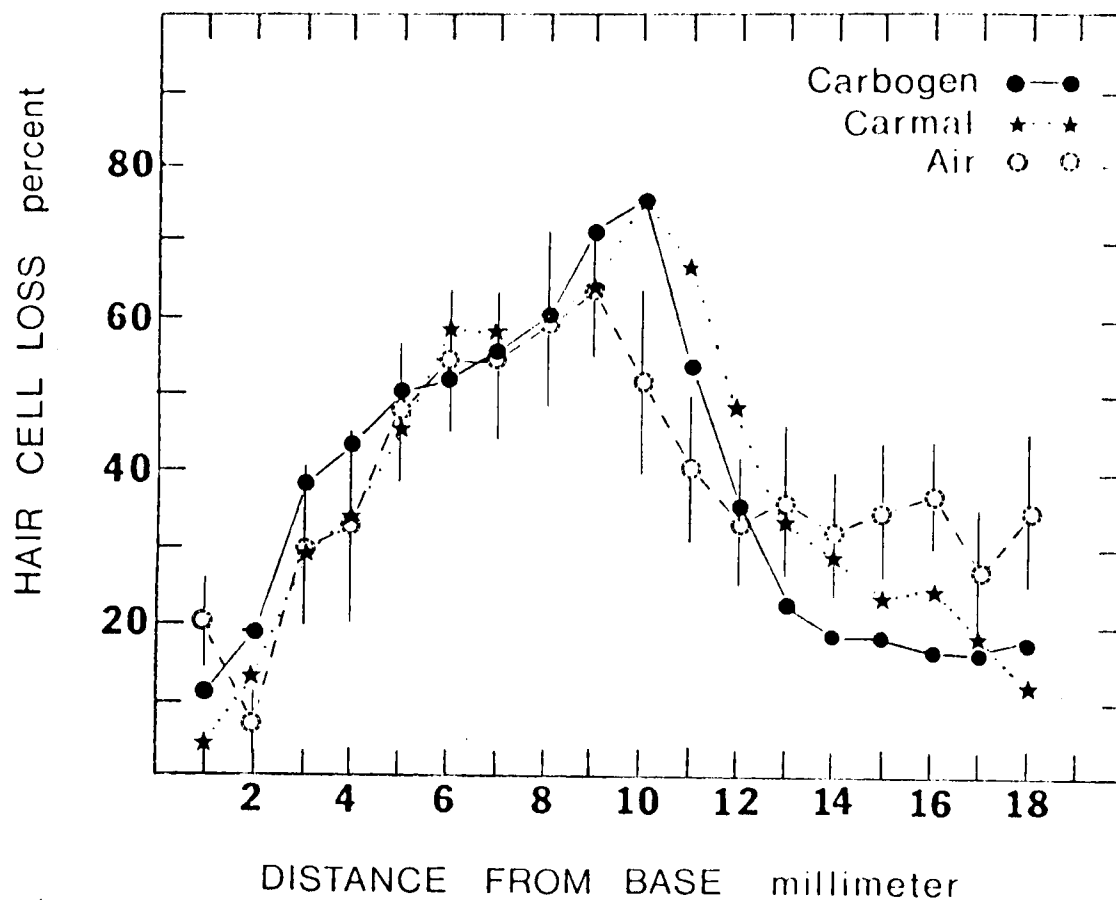


Figure A-3. Average percent total hair cell loss showing the standard error of the mean (vertical lines) for the air group.

LIST OF ABBREVIATIONS AND DEFINITIONS

ABER—Auditory Brainstem Electrical Response. ABER is an auditory evoked potential which is neural in origin.

AOAA—Amino-oxyacetic acid.

Basilar Membrane—A fibrous plate extending from the osseous spiral lamina to the spiral ligament on the outer wall of the cochlea. It separates the scala media from the scala tympani and supports the organ of Corti (ANIS, 1973).

Carbogen—A gaseous mixture of 5% CO₂/95% O₂.

Carmal—A term used in this study to stand for normal air plus extra CARbon dioxide, or 5% CO₂/21% O₂/74% N₂.

CAP—Compound action potential. Refers to the whole-nerve (acoustic) action potential usually recorded with an electrode at the round window of the cochlea. Since it is typically diphasic, it has been called the compound action potential (Eggermont, 1976).

CM—Cochlear microphonic. An a-c electric potential generated within the cochlea in response to a sound stimulus. The source of the potential is believed to be the cuticular plates of the hair cells in the organ of Corti (ANSI, 1973).

Electrical Impedance Plethysmography—A method of monitoring changes in cochlear blood flow by measuring changes in electrical impedance due to the electrical impedance difference between blood and other body fluids and tissues (Suga and Snow, 1969).

IHC—Inner Hair Cell.

MTL—Median Threshold Level. A level established in this study which was equal to the median ABER threshold level of 18 chinchillas.

Modiolus—The conically shaped central core of the cochlea. It contains the spiral ganglion of the cochlea and forms the inner wall of the scala vestibuli and the scala tympani (ANSI, 1973).

NIHL—Noise Induced Hearing Loss.

NITTS—Noise Induced Temporary Threshold Shift.

Organ of Corti—A series of neuroepithelial hair cells (receptor cells for hearing) and their supporting structures (ANSI, 1973).

OHC—Outer Hair Cell.

PTS—Permanent Threshold Shift.

Spiral Ligament—The periosteum-like outer wall of the scala media, covered by the stria vascularis (ANSI, 1973).

Scala Media—The cavity of the cochlear duct, filled with endolymph. It is separated from the scala tympani by the basilar membrane and from the scala vestibuli by Reissner's membrane (ANSI, 1973).

Scala Vestibuli—Perilymph-filled passage of the cochlear canal (ANSI, 1973).

Stria Vascularis—A vascularized epithelial structure that covers the spiral ligament within the scala media. It is thought that the stria vascularis is the source of the endolymph and of its electrical polarization within the cochlea (ANSI, 1973).

TTS—Temporary Threshold Shift.

VITA

Gary D. Harris was born to Walter and Glenna Harris on November 23, 1952, in Huntington, West Virginia. He attended elementary and secondary schools in Cox's Landing and Barboursville, West Virginia. He graduated from Barboursville High School in 1970.

In 1973 he was wed to Cheryl L. Galford and graduated from Marshall University in 1975 with his B.A. in Speech and Hearing Therapy. He went on to obtain his M.A. in Audiology at The University of Tennessee, Knoxville, in 1976.

He worked two years as a clinical audiologist in Charleston, West Virginia for Audiology and Hearing Aid Services.

Farah, his daughter, was born April 23, 1977, and then Bethany on December 22, 1978.

In 1978 he returned to The University of Tennessee, Knoxville, to work toward the doctorate degree in Speech and Hearing Science.