THE EFFECTS OF CARBOGEN INHALATION ON ACOUSTICALLY-INDUCED AUDITORY IMPAIRMENT IN THE GUINEA PIG

by

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A THESIS

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LIST OF ABBREVIATIONS

AC	Alternating Current
A/D	Analog to Digital
ANOVA	Analysis of Variance
BBN	Broad Band Noise
Carbogen	Gas Composed of 5% CO_2 and 95% O_2
dB	Decibel
DC	Direct Current
G.P.	Guinea Pig
Hz	Hertz (cycles per second)
IHC	Inner Hair Cell
MEMA	Middle Ear Muscle Activity
N_1	Cochlear Nerve Action Potential Wave #1
OHC	Outer Hair Cell
PO_2	Partial Pressure of Oxygen in Arterial Blood
PCO_2	Partial Pressure of Carbon Dioxide in Arterial Blood
PTS	Permanent Threshold Shift
RD	Recovery and Depression
RMS	Root Mean Square
RR	Relative Recovery
SD	Standard Deviation
SEM	Standard Error of the Mean
SPL	Sound Pressure Level in db re:0.0002 μbar (20 μPa)

Abbreviations (continued)

T_1	Turn 1 (Basal Turn) of Cochlea
T_2	Turn 2 of Cochlea
T_3	Turn 3 of Cochlea
T_4	Turn 4 of Cochlea
TS	Test Sequence
TTS	Temporary Threshold Shift
vas spirale	Commonly used term for Vessel of the Basliar Membrane
$\overline{X}$	Mean Value

INTRODUCTION

With technological emancipation from nature, man's ability to generate intense sound has outpaced his ability to restrict the prevalence of excessive noise. Noise pollution confronts industrialized society with yet another environmental danger. The pathological effects of this pollutant range from hearing loss to hypertension to coronary heart disease.

Despite awareness of noise as a health hazard, little conclusive evidence has accumulated concerning the actual mechanisms underlying permanent or temporary threshold shifts in hearing acuity which may result from acoustic overexposure. The occurrence of mechanical trauma to sensory and supporting structures due to sound of extremely high intensity is well documented. It is somewhat more difficult to explain how moderate intensity sound, which does not mechanically destroy cochlear sensory cells, leads to delayed degeneration of these structures often occurring at a considerable interval after the initial insult.

One relevant theory (41) (57) proposes that a metabolic alteration in sensory cells occurs as a result of a noise-induced vascular insufficiency in the inner ear. Several studies have provided evidence that noise at moderate intensities leads to a narrowing of the capillaries within the cochlea as well as decreases in the oxygen tension within the fluid compartments of the inner ear (42) (72). A noise-induced vascular insufficiency may lead to hypoxia of sensory and supporting structures of the organ of Corti compounding mechanically induced trauma due to intense sound.

Two studies (48) (108) have found that inhalation of carbogen gas (a high CO_2 and high O_2 mixture which is a vasodilator and hyperoxygenator of the blood, endolymph and perilymph) leads to decreases in the magnitude of sound-induced temporary auditory impairment. However, these studies used very small numbers of chinchillas and humans and very limited measures of cochlear function. In addition, no measurement of other physiological effects of the gas such as changes in arterial PCO_2, PO_2 or pH were attempted. Because of the limitations of the two studies, these findings may be equivocal and bear close scrutiny.

In the present studies the specific aims were to determine if carbogen inhalation during acoustic exposure would lead to changes in the magnitude of (a) permanent and (b) temporary auditory impairment in groups of guinea pigs. More complete and meaningful assessment of cochlear function was striven for using both electrophysiological and histological measures. Additionally, measurements of arterial PO_2, PCO_2 and pH were made.

Two experiments were performed. The first study was designed to evaluate the effects of carbogen inhalation on permanent cochlear damage resulting from exposure to high intensity broad band noise. Measures of cochlear integrity were performed 30-40 days after the initial insult and included the AC cochlear potential and microscope counts of missing outer and inner hair cells. The second study was designed to evaluate the effects of carbogen inhalation on temporary auditory impairment within a 3 hour interval after a moderate intensity pure tone exposure. Cochlear impairment

was evaluated using the action potential of the cochlear nerve.

The finding that carbogen inhalation leads to reductions in acoustically induced auditory impairment would lend substantial weight to the vascular insufficiency hypothesis of noise induced hearing loss. Additionally, it would indicate that this gas or agents which duplicate its physiological effects may someday become useful clinical tools.

Effects of Acoustic Overexposure on Cochlear Morphology

It has been suggested by several authors that damage due to excessive sound may occur in two ways, depending on the intensity of the exposure (36) (92). These may be generally classed as mechanical damage, and metabolic damage.

At very high intensities of sound (above 135 dB SPL*) the most commonly reported form of cochlear damage is a complete mechanical disruption of the organ of Corti. This structure may be completely thrown off the basilar membrane throughout the length of the cochlea (15) (24). With shorter durations of high intensity acoustic exposure the organ of Corti may be disrupted only at a site presumably corresponding to that of peak mechanical displacement (28). These observations indicate that a mechanical effect may be primarily responsible for cochlear damage resulting from high intensity sound. Such effects occur at the same point in time as the acoustic overexposure.

With longer exposure and sound of lower intensity, microscopic

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*SPL: Sound Pressure Level in dB re: 0.0002 μbar (20 μPa).

morphological changes include rearrangement of cellular contents, swelling of hair cells and neural dendritic processes with cell rupture. The fact that such changes appear to occur with some time lag after cessation of the acoustic exposure suggests that intracellular mechanisms of damage may be involved (36) (92) (94). While at intensities above 125 dB SPL both mechanical destruction and metabolic exhaustion may contribute to hair cell loss (92), at lower sound intensities metabolic effects probably predominate and structural alterations appear only as secondary manifestations of the metabolic disturbance.

Permanent cochlear impairment due to acoustic overexposure results from destruction of the hair cells of the organ of Corti. Damage usually begins in the OHC's then proceeds to the IHC's. Evidence suggests that the area of peak OHC loss corresponds to the frequency spectrum of the sound used for the acoustic exposure (28) (92) (94). However, such effects are not always in correspondence with the place theory of frequency discrimination (4) (13) or with Greenwood's mapping of the cochlea (26) (34). Acoustic overexposure using broad band noise (BBN) which includes a large range of frequencies affects hair cells more evenly distributed throughout the cochlea (24). In previous work by this author OHC loss due to 70 hours of exposure to BBN at 115 dB SPL occurred in sites from the base to the apex of the guinea pig cochlea (10).

Electrophysiological measures of damage due to moderate intensity BBN in guinea pigs revealed that the major depression of the AC cochlear potential occured in the 4 kHz range (39) (49). This may

correspond to the well known "4 kHz notch" in the audiograms of noise exposed humans. With longer durations of exposure to BBN the AC cochlear potentials are depressed over a larger frequency range (55) (92).

Many authors have described the course of damage within the cochlea due to excessive noise using both light and electron microscopy. Engstrom and co-workers (24) found OHC swelling, nuclear pyknosis, mitochondrial degeneration and vacuolization of cell cytoplasm as initial changes. Spoendlin (92) noted swelling of OHC's, swelling of dendrites to both OHC's and IHC's with eventual cell rupture and retrograde nerve degeneration. Lim and Melnick (61) have observed formation of blebs on the surface of the OHC's, vesiculation proceeding to vacuolization of the smooth endoplasmic reticulum, heavy accumulation of lysosomal granules and mitochondrial degeneration.

Kellerhals(49) noted that after a standardized exposure to the noise of gunshots, the number of destroyed OHC's had doubled and the number of destroyed IHC's had trebled 8-10 days after the initial insult. Such long-latency degenerative changes are consistent with a latent metabolic insult. Typically, following acoustic exposure at about 120 dB SPL, a number of cells appear to be destroyed immediately while a further number enter a slow degenerative process that may take 30 days or more for complete resolution (55). The changes observed in the cytoplasm of these cells (61) are consistent with metabolic disturbances as is the observation that isolated OHC's or IHC's may undergo such changes while neighboring cells are left intact. It has been suggested that the

eventual loss of function of an ear is determined by its ability to recover rather than by the initial insult (55).

Metabolic changes in cells would lead to disruptions in the processes necessary for the maintenance of homeostasis. Ionic imbalances, cell swelling and lysis are likely sequelae of such a deficit. Cell swelling may render cell membranes more friable than normal and further damage due to noise may result as a consequence of decreased resistance to mechanical displacement. The question is, what is the etiology of the initial metabolic lesion in the acoustically exposed cochlea?

Vascular Supply of the Cochlea

Mammalian cells depend on a continuous supply of oxygen and nutrients as well as removal of waste products. Although short term survival under anoxic conditions is possible, the majority of mammalian cells will not survive under conditions of prolonged hypoxia.

The vascular system of the organism provides a constant oxygen and nutrient supply to the cells under normal conditions. Blood vessels run in close proximity to cell borders allowing for diffusion and/or transport of substances between cell and vessel. In the cochlea of mammals and other vertebrates, however, the sensory cells exist at a relatively long distance from their blood supply. This situation contrasts with that in the vestibule where capillary networks are found beneath each sensory area. Further, the vascular network of the organ of Corti is thin and the vessels extremely

small in diameter (5 μ in the guinea pig cochlea) (2). Blood flow in such vessels is relatively slow. These anatomical factors might reasonably be expected to pose problems for sensory and supporting cells of the organ of Corti during fluctuating blood pressure or metabolic loads.

The arterial blood supply to the cochlea may be sequentially traced from the basilar artery to the anterior inferior cerebellar artery to the labyrinthine artery (87). In the guinea pig the labyrinthine artery divides into a common cochlear branch and an inferior vestibular branch. The former again divides into the posterior vestibular artery and the proper cochlear artery (2). This latter vessel enters the modiolus with the cochlear nerve. The proper cochlear artery supplies the cochlea via arteriolar branches. Some of these ascend for short distances and cross over the scala vestibuli in bony channels. There they break up into capillaries which supply the lateral wall of the cochlear duct, the spiral ligament and the stria vascularis (Figure 1). Other arteriolar branches descend to the limbus and the basilar membrane and form capillary networks on the medial side of the cochlear duct between scala media and scala tympani. These networks are in the closest proximity to the inner hair cells (IHC) and the outer hair cells (OHC) and provide the vascular bed for these structures (58).

Lateral Wall Vasculature

Smith (86) (87) and Axelsson (2) have identified several capillary beds of the cochlea and have assigned these into groups

on the basis of anatomical location. The first, on the lateral wall, is a network of capillaries within the upper spiral ligament (Figure 1). Some of these are located near the mesothelial cell layer and the perilymph. Smith (87) suggests that this network may be the most important interchange site between the blood and the perilymph of the scala vestibuli.

The second capillary bed is found running spirally within the stria vascularis. These vessels lie in the epithelia and form a dense network within the stria. The capillaries here are often larger in diameter (8-15 μ) (81) than other capillary beds of the guinea pig cochlea. As with other capillary beds of the cochlea, these vessels do not contain smooth muscle contractile elements in their walls although pericytes have been shown to be present.

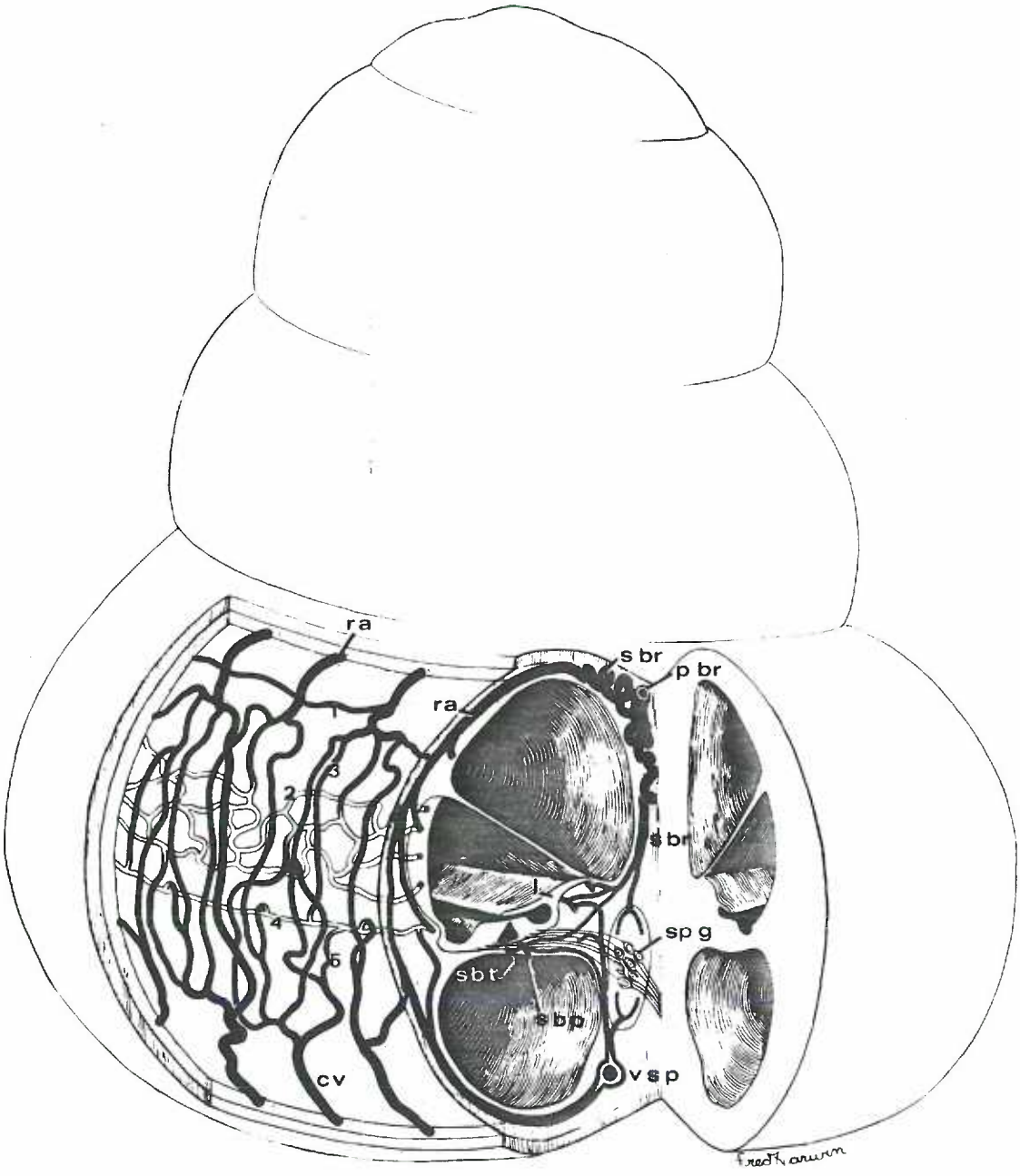
Although it has long been suggested that the stria vascularis might be a source of oxygen and nutrients for the organ of Corti, Misrahy and co-workers (70) were the first to produce evidence supporting this suggestion. They noted that the oxygen tension in the endolymph near the stria was 55-70 mm Hg whereas closer to the organ of Corti it decreased to 16-25 mm Hg. On the basis of this oxygen concentration gradient they proposed that the organ of Corti received its oxygen supply from the vessels of the stria vascularis (72). Current research has not supported this speculation (59 (60).

The third capillary group described by Smith is the arteriovenous arcades which traverse the spiral ligament from the secondary arterioles to the venules. These vessels have been found to contain contractile elements and the blood flow here is relatively

Figure 1. Schematic diagram of the capillaries in the cochlea of the guinea pig. The basal coil has been opened and a part of the bony otic capsule removed to better display the capillaries. cv:collecting venule; p.br: primary branch of the labyrinthine artery; ra:radiating arteriole; a.br:secondary branch; abp:spiral border below the inner pillar; sbt:spiral border below the tunnel; spg:spiral ganglion; vsp:posterior spiral vein.

1. Capillaries of the upper spiral ligament.
2. Network of the stria vascularis.
3. Arteriovenous arcades.
4. Capillary in the spiral prominence.
5. Capillary in the lower spiral ligament.

From Smith, C.A., Vascular Patterns in the Membranous Labyrinth (87).


ra
s br
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2
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cv
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fast. A fourth capillary network is located in loose connective tissue within the spiral prominence. A fifth network is located in the spiral ligament bordering the scala tympani.

Medial Wall Vasculature

Branches of the proper cochlear artery which supply the medial wall of the cochlea descend in the modiolus and emerge from the bone below the medial attachment of Reissner's membrane (87). These vessels form two layers. The first is a loose network within the limbus. The second is a looped network which runs between the basilar membrane and the mesothelial cells covering the scala tympani. This latter network of vessels is in the closest proximity to the tunnel of Corti and is often referred to as the vas spirale (56) or vessels of the basilar membrane (2).

Lawrence (58) has provided strong evidence that the vas spirale is responsible for the fluid and gas exchange necessary for maintenance of the hair cells. By selective surgical interference, vessels leading to the vas spirale or to the stria vascularis were occluded. Although the sample of animals in which successful occlusion occurred was few, it was found the occlusion of vessels leading to the vas spirale led to degeneration of the hair cells leaving the stria vascularis intact. Conversely, occlusion of the vessels leading to the stria vascularis caused degeneration of that structure without hair cell involvement (56). It was further observed (60) that during induced hypoxia of the organ of Corti, the fluid within the tunnel of Corti showed a decline in oxygen tension 2.3 seconds before a similar decline in the endolymph. Depression and recovery of the oxygen dependent cochlear potentials corresponded

with oxygen tension changes in the tunnel of Corti rather than in the scala media endolymph. The authors concluded (58) that the hair cells receive their oxygen from the vas spirale and not the stria vascularis as had been proposed by Misrahy (70) in 1958.

Variations in Cochlear Blood Flow

Because the capillary networks of the stria vascularis and the basilar membrane do not possess smooth muscle cells which might act in constriction or dilation of these vessels, how might changes in blood flow occur within these capillaries?

Muscular elements which may play a role in vasomotor responses have been identified in the arteriovenous arcades and in the arterioles proximal to the capillary networks. Alterations in capillary flow within the other vessels of the cochlea would be expected to occur as a consequence of changing pressures or patterns of resistance in these latter two sites. A few scattered pericytes have been found within the networks of the inner ear. Although these cells have not been considered to be contractile (78) electron micrographs have been obtained showing a spiral ligament vessel apparently constricted by the long process of a pericyte (42).

Although there is an apparent lack of vasomotor mechanisms within the capillary beds of the cochlea, adrenergic nerve fibers have been located following the cochlear branch of the labyrinthine artery into the modiolus (86). Spoendlin and Lichtensteiger (91) using a fluorescence technique found a dense network of adrenergic nerve fibers in the spiral lamina. These have been found to send

short branches to the capillary beds nearby (89). It may be suggested that blood flow variations within the microvasculature of the cochlea may be the result of systemic changes; of muscular alterations in patterns of flow in arterioles or in arteriovenous arcades; by local actions of pericytes; or by such mechanisms as capillary endothelial cell swelling. A probable consequence of vessel damage would be platelet aggregation and the formation of a hemostatic plug. Aggregated platlets resulting from endothelial cell damage would be expected to aggravate decreases in blood flow within a capillary network.

Perlman, Tsunno and Spence (82) using photographic inspection of the vessels of the stria vascularis have found a marked increase in blood velocity after administration of vasopressor agents. These changes were closely related to changes in mean carotid blood pressure. No changes in the diameter of the strial vessels were noted during the increased flow period. Matsuyama (67) found increases in flow rates in the radiating arterioles without diameter changes after administration of various drugs or carbon dioxide gas. Using electrical impedance plethysmography (based on the impedance changes between two electrodes which varies with blood volume) Morimitsu, Matsou and Suga (77) have inferred that cochlear blood flow between the scala vestibuli and the scala tympani decreased after inhalation of pure oxygen but increased after inhalation of 10% carbon dioxide gas with 90% O_2. These authors also noted that blood volume increased in response to <u>mild</u> sound. Suga and Snow (95) using the same techniques noted that inhalation of 10% CO_2 caused a 244% increase in

cochlear blood flow over control values whereas inhalation of 100% O_2 caused a decrease in flow to 57% of control values.

Sound and Cochlear Blood Flow

Observations of the ways in which the cochlear microvasculature reacts to acoustic stimulation are relevant to this study. Perlman and Kimura (81) reported that noise intensities in the range of 135-155 dB SPL resulted in a marked increase in blood flow in the stria vascularis. Although high intensities were found to halt circulation in these vessels, lower intensities of sound (e.g., 120 dB) had no effects on cochlear blood flow. The method used for measuring the rate of blood flow in the experiments described above was a direct microscopic inspection of the vessels. No change in cochlear blood flow in response to 100 dB sound has been recently reported by Hulcrantz, Angelborg and Linder (45).

Lawrence, Gonzales and Hawkins (57) using histological preparations from acoustically exposed (120 dB) guinea pigs found that the vessels of the stria vascularis and of the vas spirale were devoid of red blood cells in the areas of the cochlea corresponding to the particular frequency of exposure. Following 8 hours of exposure at 120 dB the vessels of the stria were found to be markedly narrowed (41). Changes following long exposures appeared to be caused by endothelial cell swelling. Using electron microscopy the protrusion of endothelial cells into capillary lumina was confirmed. This protrusion caused these passages to become as narrow as 1 μ (42).

More recent studies have demonstrated poor penetration of contrast medium into the vessels of the spiral lamina and stria vascularis of the chinchilla cochlea exposed to 8 hours of 100 dB noise (62). Similar results have been reported in guinea pigs using indian ink perfusion (109). Duvall, Ward and Lauhala noted a failure of horseradish peroxidase to penetrate to the small vessels of the stria vascularis during exposure to 123 dB sound (19). This effect was found to last for some time following cessation of the acoustic exposure. Kellerhals (49) has found the strial vessels of the guinea pig to be so densely packed with red blood cells after noise exposure that they appeared to form a homogenous mass. Aggregation was suggested to have occurred due to sludging and decreased flow. Recent work on effects of noise on peripheral vasculature other than the ear has reported vessel constriction in human skin (47) and the tails of rats (9).

Sound and Cochlear Oxygen Tension

Studies of oxygen tension within the fluid compartments of the cochlea have produced data which are consistent with the hypothesis that noise-induced vascular insufficiency may participate in the pathogenesis of noise-induced hearing loss. Misrahy (72) and co-workers found that the PO_2 of the endolymph decreased sharply in response to intense sound. Following pure tone bursts of 130-135 dB the drop in endolymphatic PO_2 was found to be in the range of 20%-90%. A return to normal oxygen tension was found in some animals but not in others. After sound of lesser intensity (115 dB)

the drop in endolymphatic PO_2 was less marked (10%-15%). It was suggested by the authors even at that time (1958) that part of the hearing loss induced by loud sound might be accounted for by endolymphatic hypoxia. This was the result, it was further suggested, of a diminution in the blood supply or by an increase in metabolism within the active elements of the cochlea.

Koide (53) has more recently reported decreases in the oxygen tension of the perilymph and cerebral cortex of both rabbit and guinea pig during exposure to test tones (approximately 105 dB SPL) but not during exposure to shock waves.

The first cellular metabolic alteration which would be predicted to occur as a result of an oxygen deficiency would be an interruption of oxidative phosphorylation, the aerobic form of energy production. It is suggested that the changes in the metabolic status of the sensory cells of the cochlea, primarily that of oxidative metabolism, may be a contributing factor in noise-induced hearing loss.

Metabolism in Cells of the Cochlea

The cochlea is a very active site of oxidative physphorylation. Early measures of the respiration rates of different tissues based on the rate of consumption of oxygen (12) revealed that the stria vascularis was extremely high (10 μl O_2/μl tissue/hour). This level of O_2 consumption was significantly higher than that found in the saccule or the utricle (3.8 and 2.4 μl O_2/μl tissue/hour respectively). The oxygen consumption in the latter two sites was

generally comparable to that in other areas considered to be very metabolically active such as proximal and distal renal tubules (3.6 µl O_2 /µl tissue/hour) or choroid plexus (5.6 µl O_2/µl tissue/hours). The stria has a very high requirement for oxygen when compared to the other tissues of the body, presumably due to its role in the production of endolymph (86).

It has been found that the cells of the organ of Corti are specialized to participate in anaerobic energy production compared to those in the stria vascularis. Matchinsky and Thalmann (64) (65) using a method based on the fluorescence of pyridine neucleotides analyzed material from the stria vascularis and from the organ of Corti. They found 3 times more glycogen and 10 times more glucose-6 phosphate (an intermediate in glycolysis) in organ of Corti as compared to the stria. The activity of the glycolytic enzyme glycogen phosphorylase was 5 times higher in organ of Corti. In contrast, the citric acid cycle enzymes citrate synthase and malate dehydrogenase were 2.5 times higher in the stria than in the organ of Corti. These findings indicate that while the organ of Corti as well as the stria vascularis are dependent on aerobic metabolism, the organ of Corti appears to have more capacity for glycolysis than does the stria (66). In that the organ of Corti may be further from ready sources of oxygen or that the rate of blood flow to the cells of this structure may undergo large variations, this capacity would seem to be both necessary and adaptive.

Schneider (85) found that levels of lactate in perilymph increased during noise exposure, suggesting that anaerobic forms of

respiration may predominate during this time. Ishii, Takohoshi and Balogh (46) found that after exposure to BBN (110 dB SPL for 30 min) the levels of glycogen in the OHC's of the guinea pig decreased substantially. They suggested that the relatively larger stores of glycogen in the organ of Corti were being utilized during sound exposure due to cellular hypoxia.

The decline in the endolymphatic pO_2 during exposure to loud sound (72) and the biochemical changes in the organ of Corti may indicate (a) that a vascular insufficiency has developed that is limiting the delivery of oxygen to the cochlea or (b) that the transduction of sound into nerve impulses is an energy-consuming or at least an oxygen-consuming process.

Studies of the bioelectric potentials of the ear during systemic or local hypoxia indicate that oxygen is a prerequisite for their maintenance as well as the maintenance of all cell processes. In view of the microvascular anatomy of the cochlea and its responses to loud sound (57) it appears that the availability of oxygen and nutrients to the sensory cells of the organ of Corti may be limited during acoustic exposure when they are most needed. That is, restriction of blood flow as a consequency of acoustic overstimulation may be coupled with increased requirements of cells for the oxygen necessary to fuel the energy processes that transduce that sound. In summary, noise may be viewed as a metabolic stressor of the ear in two, separate but possibly synergistic, ways. These are, by increasing the requirement for oxygen and nutrients and by decreasing the delivery of those items by the induction of a vascular insufficiency.

Effects of Cochlear Hypoxia

The effects of hypoxia on cochlear potentials and inner ear structure have been well described. All forms of hypoxia, whether induced by vascular occlusion or by asphyxia, quickly abolish the nerve action potentials and reduce the AC cochlear potentials to a small fraction of their initial value (60). Oxygen tension of the endolymph and the perilymph falls quickly during anoxia induced by breathing pure nitrogen (54). The amplitudes of the action potentials decrease 22 seconds after the initial drop in endolymphatic pO_2 and disappear completely within 1 minute (72). The AC cochlear potentials begin to decrease 6-7 seconds after the action potentials and reach their minimum value in 1.5 minutes. Both of these cochlear potentials may regain their initial value if restoration of normal air occurs quickly. After prolonged hypoxia (6-22 minutes), action potentials and AC cochlear potentials may never return to normal values (71).

The changes in cochlear potentials during hypoxia were described by Bekesy (4) and by Wever and co-workers in the early 1950's (106). Lawrence (60) found that during respiration of deoxygenated air, the AC cochlear potentials of cats were not affected until mixtures containing less than 4% O_2 were used. Further reductions in respired oxygen caused further declines in AC cochlear potentials. Potentials recovered only partially with restoration of normal air. Repeated anoxic exposures led to a cumulative deterioration of response.

The endolymphatic potential and summating potential of the

cochlea show similar depressions during hypoxia but usually return to full normal levels. Although these potentials seem more resistant to hypoxia, the AC cochlear and the action potentials are very much less so (11).

Lawrence and Wever (54) using microscopic inspection of the histological preparations of cat ears found that following cochlear hypoxia the supporting cells showed the earliest morphological damage while the structure of the hair cells seemed to be preserved. After more prolonged hypoxia the hair cells became involved as well. The basal end of the cochlea always showed more extensive degeneration than did the apical end. An additional observation was that the degree of depression of the AC cochlear potentials in these ears depended on the method used for induction of the hypoxia. During anoxia induced by respiration of deoxygenated air, the depression of the AC cochlear potentials was on the order of 13 dB. After a more violent death induced by tracheal clamping and asphyxia, however, the depression of the AC cochlear potential was on the order of 34 dB. This indicates that the ear may have a certain metabolic reserve that may be decreased depending on the systemic requirements for consumption of oxygen.

Falbe-Hanson and co-workers (25) found no morphological changes in the organ of Corti in guinea pigs or cats after prolonged hypoxia induced by tracheal clamping even though the AC cochlear potentials had been permanently reduced. Kimura and Perlman (52) used obstruction of the labyrinthine artery to induce cochlear ischemia in guinea pigs. By sacrificing of the animals at sequential intervals

following the arterial obstruction an orderly sequence of histological changes could be established for all cochlear structures. The hair cells were found to be highly vulnerable. Within three hours the IHC's were fully disintegrated while OHC's were still present although damaged. Within six hours all hair cells were destroyed. Supporting cells had also disintegrated within this time interval. The stria vascularis also appeared to be highly vulnerable to ischemia. This structure began detachment from the spiral ligament within one hour and began to disintegrate within four hours. Thus it appears that the hair cells, supporting cells and the stria vascularis are the most vulnerable sites in the cochlea for damage due to hypoxia.

Bohne (8) found that ischemia of the cochleae of chinchillas by incubation of the temporal bones in a nitrogen atmosphere for various lengths of time prior to fixation induced a variety of pathological changes. After 30 minutes of this treatment, nerve fibers were swollen as well as the IHC's and OHC's. After one hour all hair cells were disrupted in pattern. After two hours severe degenerative changes were present in both nerve fibers and hair cells.

It has been found that during hypoxia the energy reserves of the different structures decline at different rates. Matchinsky and Thalmann have found that energy reserves declined most rapidly in the stria vascularis followed by the spiral ganglion, auditory nerve and the organ of Corti (66) (96). In all cases glycogen was the slowest substance to change and phosphocreatine, the storage

form of ATP, was the fastest. The rate of degradation of glycogen occurred most rapidly in the basal turns of the cochlea and most slowly at apical levels. The authors have concluded that while all inner ear structures are dependent on oxidative metabolism, the organ of Corti possesses larger carbohydrate stores and hence a larger potential for anaerobic respiration (96). Such alterations in biochemistry of the organ of Corti as increased lactate production (85) and decreased stores of glycogen (46) resulting from hypoxia would lead one to predict that the organ of Corti may be specialized to survive hypoxia. However, under larger stresses or over long periods, anaerobic energy reserves may not be capable of maintaining cell function.

In a classic paper bearing on this, Tonndorf, Hyde and Brogan (97) examined the reductions in the AC cochlear potentials due to acoustic overexposure in normal and in hypoxic guinea pigs. Their hypothesis was that the double stress of noise and hypoxia would lead to comparatively larger shifts in these potentials than would noise alone. This prediction was confirmed. A 1 kHz tone at 130 dB was presented for 1 minute in conjunction with respired oxygen concentrations of 21% (normal air), 10% and 8%. It was found that both the decline and the recovery of the AC cochlear potentials was influenced by the hypoxic state. Potentials decreased more rapidly and to lower levels and recovered more slowly at 8% O_2 than at 10% and more slowly at 10% O_2 than at 21% O_2. It thus appears that an adequate supply of oxygen is a prerequisite for optimal recovery from acoustic overstimulation.

Effects of Cochlear Hyperoxia

In contrast to the number of studies dealing with the effects of oxygen deprivation on cochlear function, relatively few investigations have been made into the effects of increasing the supply of oxygen to the cochlea.

Kellerhals (49) used low molecular weight dextran in guinea pigs in an attempt to increase blood flow and decrease sludging of blood within the inner ear during acoustic trauma. Histological examination of the IHC's and OHC's of guinea pigs and the result of clinical trials in humans was reported to confirm the favorable effect of such treatment on recovery from acoustic trauma. It was suggested that these effects were the result of increases in blood flow to cells that had been metabolically impaired rather than to cells which had been mechanically damaged by the sound. Study of the use of low molecular weight dextran in the treatment of acoustic trauma in the guinea pig at this laboratory has not confirmed its effects on cochlear damage due to acoustic overstimulation (100).

Other agents which exert effects on the oxygenation of the blood or on the flow of blood to or within the cochlea have been investigated (82) (90). Suga and Snow (95) used electrical impedance plethysmography to measure blood volume while simultaneously recording the AC cochlear potentials from the basal turn of the cochlea resulting from a 1 kHz tone at 80 dB. With inhalation of pure oxygen, blood volume decreased to 57% of control values. In contrast to the effects of pure O_2 inhalation, inhalation of 10% CO_2

and 90% O_2 caused blood flow increases to 244% of control values. Following 15 minutes of respiration of this gas, cochlear blood flow stabilized at 333% of control value (95). Cochlear potentials were found to be stable during administration of the gas as long as the general condition of the animal remained good. Analogous results have been described for human cerebral blood flow by Kety and Schmidt (50). These authors concluded that in all cases alterations in peripheral resistance precipitated the changes in blood flow. Prazma and co-workers (84) have described an increase in the PO_2 of the endolymph to 101% over normal values after 20 minutes of respiration of 10% CO_2/90% O_2 in the guinea pig.

Measurement of human perilymphatic pO_2 using a polarographic principle has been accomplished by Fisch, Murata and Hessli (27). Carbon dioxide and oxygen caused a marked increase in oxygen tension in perilymph. Carbogen gas, composed of 5% CO_2 and 95% O_2, produced an elevation of perilymphatic PO_2 to 200% of control values within 30 minutes. Carbogen inhalation produced little or no blood pressure elevation. Tsunno and Perlman (98) have more recently found similar increases in guinea pig perilymphatic PO_2 using 8% CO_2 in air.

A favorable effect of elevated arterial PO_2 on depression of AC cochlear potentials in the guinea pig due to ethacrynic acid has been described by Prazma and Pecorak (83). Elevation of the arterial PO_2 was found to prevent the decline in these bioelectric potentials that was observed in those animals with normal or lowered arterial PO_2. It was suggested that the elevations in

oxygen tension acted to preserve the ion transport systems affected by ethacrynic acid.

Effects of gas inhalation on noise-induced hearing loss have been examined by David Lipscomb and his group at the Noise Research Laboratory in Knoxville, Tennessee (44) (108) (48). Joglekar and Lipscomb (48) investigated the effects of inhalation of pure O_2, of carbogen (5% CO_2/95% O_2) and of normal air on the temporary threshold shifts (TTS) induced by a 1 kHz 100 dB pure tone presented for 10 minutes. Humans and chinchillas were used and conventional and behavioral audiometry were the measure of hearing acuity. The various gas mixtures were presented to different groups of animals (a) during the 10 minutes of sound exposure or, (b) for 30 minutes following the sound exposure. Recovery curves for both the pre-stimulatory and the post-stimulatory gas inhalation conditions revealed distinct differences between the groups exposed to the different gas mixtures. Inhalation of normal air led to the largest TTS in both species. Pre-stimulatory inhalation of carbogen led to the smallest TTS in both species while the groups that received pure O_2 showed intermediate effects. In the groups receiving the gas mixture following acoustic exposure similar changes were observed; the fastest recovery was seen in the group exposed to the carbogen, the slowest in the group which received normal air.

In the chinchilla population, recovery from TTS in those animals which received normal air was incomplete even 72 hours after stimulation whereas in the group that received the carbogen the TTS

was 34 dB less severe and recovery was complete within 48 hours. In the animals which received gas exposure after acoustic stimulation, those which received normal air demonstrated an apparent permanent threshold shift (PTS) after 15 days of recovery. In contrast, the group which received carbogen following acoustic stimulation, full recovery was found in 3 days. No consistent OHC damage was found for any of the groups of animals.

The limitations of this study are that only 6 animals were used in the experiment in which the gas was given during acoustic exposure and 4 animals were used in the experiment in which gas was presented after exposure. Thus there were only two chinchillas in each experimental group. Further, audiometry was only performed at one frequency (2 kHz). An additional limitation was that the acoustic trauma did not produce OHC damage and no histological data was obtained for comparison with audiological measures.

In more recent investigations (108) the Noise Research Laboratory group has demonstrated the effects of carbogen inhalation given at various times before acoustic exposure in humans and in chinchillas. Humans were exposed to a 100 dB 1 kHz pure tone. Again TTS was tested only at 2 kHz. Reduction of the magnitude of the TTS and therefore the time to full recovery were produced by carbogen inhalation. In the chinchillas, amplitude reduction of the cochlear nerve potential was used an an index of cochlear impairment. Acoustic exposure was a 1 kHz pure tone presented at 120 dB for 10 minutes. N_1 responses to 2 kHz tone bursts were measured up to 72 hours after acoustic exposure. It was found that

respiration of carbogen for 30 minutes prior to the acoustic overstimulation led to large decreases in the level of, and time to recovery from, N_1 response depression. No beneficial effects were noted if the gas preceeded the acoustic exposure by more than 90 minutes. Histological analysis of the cochleae of these animals supported the electrophysiological finding. For the animals which received normal air, the average number of missing OHC's was found to be 4.5% primarily within the basal turns of the cochlea. In the group which received carbogen one hour before sound exposure the average loss was only 0.2%. Due to small individual experimental group size it is not known if this finding represents a statistically significant difference. This study again reflects several limitations due to small group size. No attempt was made to match animals for sensitivity to acoustic exposure in order to make meaningful comparisons between groups and the N_1 responses were only measured at one frequency. In each of the two studies above no measurements of arterial blood gas or pH changes resulting from the carbogen inhalation were performed.

In several European studies alterations in blood oxygen have been correlated with several aspects of auditory impairment. Berndt (6) has found that guinea pigs which have been adapted to a simulated altitude of 10,000 meters showed less depression of the AC cochlear potential 24 hours after a 130 dB exposure than non-adapted animals. Berndt (7) also showed that an increase in metabolic rate (hyperthyroidosis) led to a larger degree of noise-induced damage than a decrease in metabolic rate (hypothyroidosis).

Giger in a prospective study (32) treated 55 cases of sudden deafness in humans with carbogen inhalation and noted an audiometric improvement one year later in the experimental group.

Toxic Effects of Oxygen

The use of oxygen as a treatment is not always beneficial to auditory function. Pure oxygen has been found to have detrimental effects that are not seen with carbogen use. Hou and Lipscomb (44) measured action potentials with round window electrodes under differing gas inhalation conditions in chinchillas. When animals were given pure O_2 two effects were noted: (a) a fast reversible depression of the action potentials, and (b) a more slowly developing chronic depression of the AP with a latency shift following multiple hyperoxic episodes. It was suggested that CO_2 depletion was the probable cause of the fast reversible depression and that the secondary effect was due to an intracellular accumulation of superoxide radicals and consequent toxicity.

Superoxide radicals are the normal product of the biological reduction of molecular oxygen (29). A family of enzymes, the superoxide dismutases, protect against the toxic effects of the superoxide radicals by catalyzing the dismutation of superoxides to hydrogen peroxide and O_2. High levels of oxygen in a system exceed the ability of the superoxide dismutases, however, and therefore may be cytotoxic.

Other detrimental effects of oxygen on auditory function have been noted by Bennett, Ackles and Cripps (5). In this

investigation, the effects of hyperbaric oxygen inhalation on auditory evoked responses was measured in military diving personnel. For subjects breathing compressed air at 10 atm. the evoked responses were severely depressed but returned to normal after decompression. The same decrement was noted at 2 atm. with respiration of pure O_2 suggesting the decrease was caused by the hyperbaric oxygen in both cases. The speed of onset of the detrimental effects suggested that this depression was not related to the formation of superoxide radicals.

Measures Used in the Following Studies

There are several electrical potentials which may be recorded from the cochlea. These include the action potential of the auditory nerve (hereafter referred to as N_1), the AC cochlear potential, the summating potential and the endocochlear potential. All except the AC cochlear potential are direct current potentials (107). Each of these bioelectric potentials can be used as a source of information concerning the functional state of the cochlea.

Both the AC cochlear potential and the N_1 potential may be measured from an electrode contacting the round window membrane. The AC cochlear potential is a relatively faithful representation of the frequency and intensity of the acoustic stimulus. The amplitude of this potential is linearly related to the amplitude of the acoustic stimulus within the majority of the range of hearing of a normal ear. The maximum electrical output of the AC cochlear potential represents a relative measure of the maximum

output of the cochlea. The AC cochlear potential is thought to have its source primarily in the outer hair cells (14). Recording of the AC cochlear potential from the round window membrane is however considered to provide a view of the activity of these cells which are closest to the basal end of the cochlea.

The cochlear nerve potential, N_1, is thought to represent the summed activity of the dendritic processes, axons and nerve cells of the primary afferent fibers of the cochlea (16) (51). Because of this, the N_1 response reflects not only the number of but also the synchrony of events at these sites. Thus the amplitude of the N_1 response depends on frequency, intensity, duration and rise/decay time of the acoustic stimulus (21). Because the majority of afferent fibers from the cochlea arise from the inner hair cells, the N_1 potential presumably represents primarily the activity of these cells. N_1 response threshold has been found to be a sensitive indicator of temporary auditory impairment (43) (73) (74). It is possible to measure the N_1 response at a large range of frequencies using tone bursts (20) (76). Due to frequency-following of the cochlear nerve fibers N_1 potentials are difficult to measure below about 2 kHz. The predominant frequency of the N_1 response itself is approximately 1 kHz.

Attempts to correlate hearing losses with changes in the AC cochlear potential have met with mixed success (40) (63) (104) (3). Good correlation has been found between <u>relative</u> depression of the AC cochlear potential and OHC loss (10) (102), between site of OHC

loss and frequency specific depression of AC cochlear potential (102) and between depression of AC cochlear potential and behavioral audiometric measures (103).

The N_1 potential is recognized as a more sensitive indicator of auditory impairment than the AC cochlear potential (16)(51)(104)(75)(76). The frequency of maximum depression of the N_1 potential due to pure tone acoustic overstimulation is found to be 1/2 to one octave above the stimulus frequency (76)(69).

Arterial blood gas values are affected by a variety of physiological and environmental factors. These include inspired gas content, rate and depth respiration and metabolic factors. Respiration of gas containing a large amount of CO_2 would be expected to increase the rate and depth of respiration and reduce the arterial pH (33).

A definitive study of arterial blood gas and pH values in the guinea pig has not been performed. Lall and Buckner (111), sampled carotid arterial blood in 10 unanesthetized guinea pigs in which a mean pH was 7.47, PCO_2 was 28.6 mm Hg, and PO_2 was 79.2 mm Hg. They also found large differences in these values from day to day. The Biology Data Book of the Federation of American Societies for Experimental Biology (1) lists mean values at pH 7.35, PCO_2 30 mm Hg and PO_2 70 mm Hg (95% O_2 saturation). Data previously collected at this laboratory* from guinea pigs recovering from anesthesia were pH 7.38, PCO_2 30 mm Hg and PO_2 85 mm Hg. The unanesthetized guinea pig is known to respond hyperactively to handling. It may be suggested that arterial blood gas values might reflect respiratory changes occurring in response to experimental stimulation.

*Mary B. Meikle, personal communication.

METHODS

In order to determine the effects of carbogen inhalation on both permanent and temporary acoustically-induced auditory impairment two separate experiments were performed. Experiment I utilized the AC cochlear potential and microscope counts of missing inner and outer hair cells as measures of permanent cochlear impairment. Measurements were obtained 30 days following an acoustic exposure which consisted of high intensity broad band noise. Experiment II utilized the amplitude of the N_1 response to tone bursts as measures of temporary auditory impairment within a 3-hours interval following exposure to a moderate intensity pure tone. Measures of arterial blood PCO_2, PO_2 and pH were obtained from animals exposed to each of the experimental conditions.

EXPERIMENT I - Effect of Carbogen Inhalation on Noise-Induced Permanent Cochlear Impairment

Animal Selection and Experimental Treatment Groups

Thirty healthy pigmented guinea pigs within a 200-300 gram weight range were selected from the stock maintained at the Kresge Hearing Research Laboratory (102). Each animal was shown to possess auditory function by exhibition of the Preyer pinna reflex. Animals were assigned into experimental treatment groups as follows:

TABLE 1

Group	No. of Animals	Acoustic Exposure	Gas Exposure
Compressed Air/120	10	120 dB SPL*	Compressed Air
Carbogen/120	10	120 dB SPL	Carbogen
Control A	5	50 dB SPL	Compressed Air
Control B	5	50 dB SPL	Carbogen(95% O_2 and 5% CO_2)

*Re: 20 μPa

Once a day over 5 consecutive days each group of animals was placed into a wire mesh cage contained within an airtight box and allowed to breathe one of two gas mixtures for 4 hours. During the last 3.5 hours of this period one of two sound exposures was continually presented to each group of animals. Upon cessation of the acoustic stimulus and gas exposure each group was removed to their home cages and allowed to breathe normal room air.

The acoustic exposure consisted of a broad band of noise at an intensity of either 50 dB or 120 dB SPL. Animals were free to roam about the wire mesh cage at all times. The gas exposure consisted of either compressed normal air or compressed carbogen (5% CO_2/95% O_2) allowed to enter the airtight wooden box at a flow rate of 15 liters/minute during the time the animals occupied the wire mesh cage.

Sound Exposure Cage

The sound exposure cage consisted of an 0.64 mm wire mesh cage

(78 cm x 35 cm x 35 cm) within a plywood box (88 cm x 45 cm x 45 cm) with a hinged top. This arrangement was constructed to provide precisely 5 cm of open space between the walls of the wooden box and the border of the wire mesh cage at each point. This minimized the effect of clustering of the animals at any point in the wire mesh to decrease the level of sound exposure. The top of the wooden box was sealed with compressible weather stripping and luggage clamps.

With the top of the cage closed the only gas access to the wooden box was provided by a 1.6 cm aluminum manifold along the rear wall. The manifold was punctured by small holes along its length within the cage to allow even entry of gas into the chamber. Air outlets were placed near the top of the wooden enclosure. A small hole in the front wall of the exposure cage allowed sampling of gas content within the chamber. The entry manifold was connected to compressed gas cylinders by 1.2 cm plastic tubing. The outlets were connected to the exterior of the building (7 meters) by 2.0 cm plastic tubing. The small O_2 analyzer tubing opened within the wooden box at animal head height. All junctions, corners, fittings and holes were sealed inside and out with silicone-rubber caulking to render the wooden enclosure airtight with the top closed.

Gas Exposure

Gas was provided by large gas cylinders (size H) supplied and analyzed by the Airco Company. Four cylinders of each mixture of gas provided an adequate supply for the entire experiment. Gas

mixtures used were either carbogen (CO_2 equal to 4.98%, 4.95%, 4.89% or 4.79% balanced with pure O_2) or normal air (CO_2 equal to 0.03%, O_2 equal to 20.95%, N_2 equal to 78.8%). Gas supplied in the cylinders was dehydrated in nature. No humidification of the gas was provided between the cylinder and the animals.

Gas flow rate was controlled by a precision 2 stage regulator designed for each type of gas (Airco) in series with a Melco flow meter calibrated for small flow rates.

Flushing of the chamber with the experimental gas was done each day before placement of the animals within the chamber. Very high flow rates of gas sufficed to bring the 190 liter wooden box to 90-95% O_2 concentration using carbogen. O_2 concentration using normal air of course remained unchanged. When the O_2 concentration in the wooden box reached 90% or after an equivalent amount of time using normal air the animals were quickly placed within the chamber and the flow rate immediately reduced to 15 liters/minute where it remained for the duration of the gas exposure period. Using carbogen, a measured O_2 concentration of 95% was found in all cases approximately 15 minutes after placement of the animals within the cage.

Because of relative ease of measurement, oxygen concentration was used as a measure of overall gas concentration within the chamber rather than CO_2 concentration. O_2 concentration was measured using a MIRA OXA-100 oxygen analyzer. This instrument was calibrated using room air and carbogen before each 5-day exposure period.

Acoustic Exposure

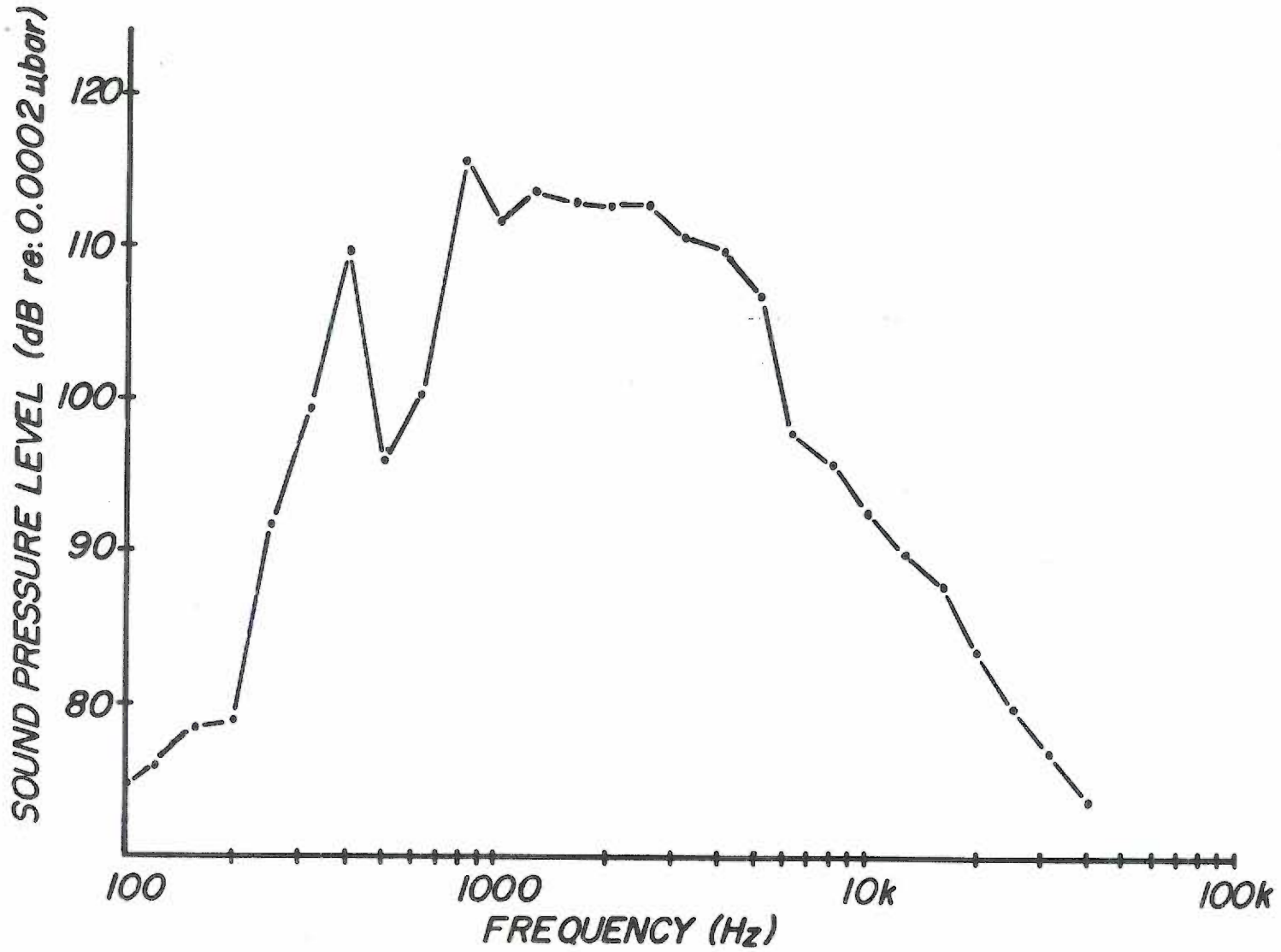
The acoustic exposure consisted of a broad band of noise presented at 50 dBC* or 120 dB linear. The frequency spectrum of the 120 dB noise measured in 1/3 octave bandwidths is shown in Figure 2. This spectrum was measured at the center of the wire mesh cage at approximately animal head height (3 cm). It may be seen that the majority of sound pressure is at frequencies between 800 Hz and 4000 kHz and drops substantially in energy beyond these points.

The 120 dB acoustic exposure was chosen as an attempt to provide a high level of metabolic stress to the ear. It was expected that this level would not provide mechanical trauma as the primary insult, yet would provide significant cochlear pathology. The low intensity acoustic stimulus (50 dBC) was well below the ambient noise level in the guinea pig colony and was added to provide an innocuous sound environment as a procedure control.

Noise was produced by a pair of JB Lansing compression drivers (No. 2482). These were mounted directly to the wooden box at the center of each of the ends. A 5 cm hole in the wooden box was aligned with the 5 cm throat of the compression driver. The speakers were wired in parallel and driven by a single channel source provided by a random noise generator (General Radio No. 1382) in conjunction with a Mackintosh 240 watt power amplifier. The amplifier power source was controlled by an electronic timer which was set to go on 30 minutes after the animals were placed within the exposure cage and to stop exactly 3.5 hours later. Sound

*The C scale is a weighted measure of sound pressure which descriminates against very high and very low frequencies.

Figure 2. One-third octave bandwidth frequency power spectrum of the broad band of noise used as acoustic exposure in Experiment I. Measurements were made at the center of the sound exposure cage 3 cm above the floor of the wire mesh cage at a sound pressure level of 120 dB. Data points are at the center frequency of each one-third octave. Note single sound pressure maximum at 250 Hz and more broad sound maximum above 800 Hz.


SOUND PRESSURE LEVEL (dB re: 0.0002 μbar)
120
110
100
90
80
100
1000
10k
100k
FREQUENCY (Hz)

pressure levels were adjusted by alterations in the output of the random noise generator.

Sound pressure levels were measured with a precision sound level meter (Bruel & Kjaer No. 2209) on the linear scale for the 120 dB sound. It was necessary to use the "C" scale for measurement of the 50 dBC noise to overcome signal-to-noise overload on the linear scale due to low (<100 Hz) frequency vibration. For calibration of the 120 dB sound field, measurements of sound intensity were made at 5 cm intervals over the entire cage floor at heights of 2.5 cm, 7.6 cm and 12.7 cm (315 points). The sound pressure averaged over all these points was 120.26 dB (standard deviation 0.9 dB). The low intensity sound was adjusted to produce 50 dBC at the center of the exposure cage but was not measured over the entire sound field.

Experimental Procedure

Animals were removed from their home cages and placed into either normal air or into carbogen atmosphere (already described) each day for 5 consecutive days. Each group of animals first was allowed to remain in the gas environment for 30 minutes with no experimental sound exposure. After this time an acoustic stimulus was introduced which continued for 3.5 hours during continuous gas exposure. At the end of the 4-hour exposure period each group was returned to their home cages. Water was available each day in both the sound exposure cage and home cages. Food was only available in the home cages.

Each animal was weighed before exposure on the first of the 5-day period, on the 3rd day and on the 5th day. (APPENDIX A) At the end of the 5-day experimental period for each group of animals an interval of 30-40 days was allowed in order to permit damage effects to stabilize. Animals were kept in their home cages during this time with full access to food and water. No further exposure to gas or to sound other than the ambient level within the guinea pig colony was given during this time. At the end of the stabilization period each animal was prepared for electrophysiological and histological evaluation of auditory function.

Preparation for Electrophysiological Evaluation

Each animal was prepared for recording of the AC cochlear potential at the round window membrane as follows. The animal was anesthetized by an intraperitoneal injection of allobarbitol (60 mg/kg) with urethan (240 mg/kg). The neck and ear areas were clipped, the trachea cannulated and the animal placed on artificial ventilation. The pinnae were excised bilaterally. A heating pad was used to maintain a rectal temperature of 38^{o} C $\pm$ 2^{o} C.

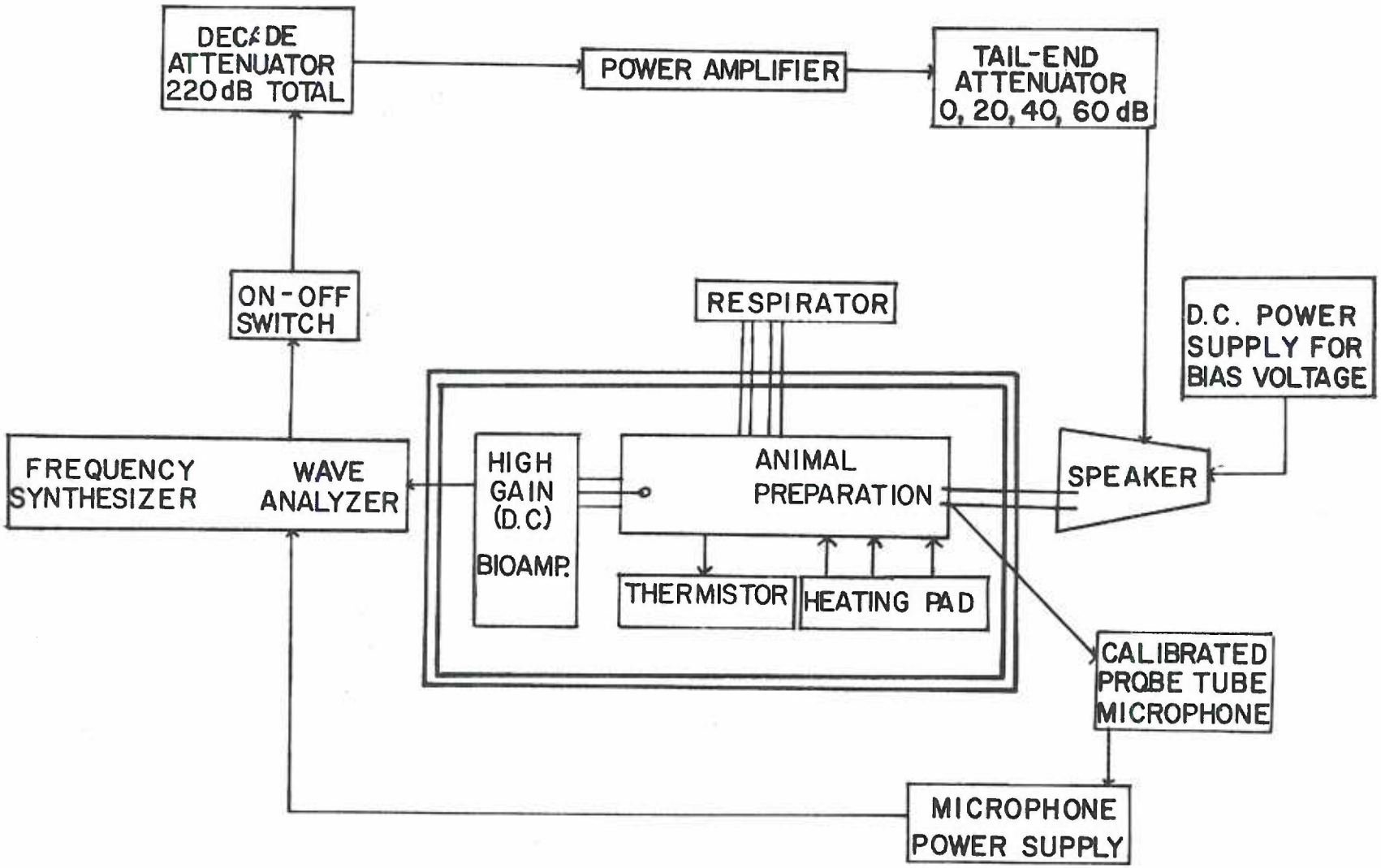
With visualization through an operating microscope, the thin muscle layer overlying the bulla was cleared by blunt dissection. With a pointed scalpel blade a small opening was made in the soft bone of the bulla; this was enlarged until a good view of the round window was provided. Bone chips and blood were removed from the site and the entire area was kept clean and dry. The animals were then secured to a small platform and this in turn secured

with magnetic clamps to the operating table.

For presentation of the acoustic test stimuli to the ear, a sealed sound system was employed. A specially designed sound cannula (99) was sealed into the external auditory meatus with silicone grease. The cannula contained provision for a calibrated microphone (Bruel & Kjaer, 1/2 inch) which terminated in a calibrated probe tube (1 mm). The sound cannula was connected to a Western Electric 555 loudspeaker by 25 cm of soft rubber tubing and 35 cm of rigid-walled tubing. The microphone was inserted into the sound cannula so that the probe tube barely projected into the external auditory meatus. This was accomplished before the test procedure and left in place during all subsequent electrophysiological measures in order that measurement of the sound intensity could be done without disturbing the connection of the ear to the loudspeaker. The sound pressure at each test frequency was measured immediately after completion of the last electrophysiological measure.

For recording of the AC cochlear potential, a silver electrode with a round ball tip was advanced by micromanipulator to contact the round window membrane. A second active electrode was inserted into the muscle adjacent to the bulla. A reference electrode was placed into the hind paw. Signals from the round window electrode were led to a battery powered, high gain differential amplifier (Princeton Applied Research) where they were amplified 1000 times and led to a narrow band wave analyzer (General Radio No. 1900-A). Figure 3 provides a schematic diagram of the entire preparation.

Figure 3. Schematic diagram of the sound producing system, animal and biological recording system used for the electrophysiological measurements in Experiment I. The active, reference and recording electrodes are not labeled. Note that the animal preparation, bioamplifier and animal warming system are enclosed within an electrically isolated, double-walled, sound shielded chamber.


DECADE ATTENUATOR 220dB TOTAL
POWER AMPLIFIER
TAIL-END ATTENUATOR 0, 20, 40, 60 dB
ON-OFF SWITCH
RESPIRATOR
D.C. POWER SUPPLY FOR BIAS VOLTAGE
FREQUENCY SYNTHESIZER
WAVE ANALYZER
HIGH GAIN (D.C) BIOAMP.
ANIMAL PREPARATION
SPEAKER
THERMISTOR
HEATING PAD
CALIBRATED PROBE TUBE MICROPHONE
MICROPHONE POWER SUPPLY

Electrophysiological Measures

Acoustic test stimuli consisted of pure tones at the frequencies of 0.1, 0.2, 0.31, 0.5, 0.7, 1, 1.5, 2, 4, 5, 7, 10, 15 and 20 kHz. A measure of the amount of sound required to bring the electrical output of the ear to 1 μV RMS at each of the test frequencies was recorded. After measurement of the actual sound pressure in each ear, a 1 μV isopotential sensitivity function for each ear of each animal could be plotted.

Additionally, a measure of the magnitude of the AC cochlear potential resulting from a sound stimulus of increasing intensity (in 5 dB steps) was recorded at 1 and at 10 kHz. Sound intensity at each of these frequencies was increased until the maximum electrical output of the ear was reached. From these measures intensity functions were plotted for each ear with a record of the maximum electrical output of the ear. All electrophysiological measures were recorded in RMS voltages. Tests were periodically made for radiation artifact from the loudspeaker by removal of the DC bias thus decreasing the sound intensity without disturbing the electrical radiations from the loudspeaker.

Histological Measures

Immediately following the last electrophysiological and sound measurement, each animal was prepared for histological examination of the cochlea using the surface preparation technique (23).

While still anesthetized the animal was decapitated, the temporal bones removed and the cochleae perfused thoroughly with

Dalton's solution containing 1% OsO_4 as a fixative. The temporal bones were then allowed to remain in the fixative for an additional 2 hours. The cochleae were then washed in normal saline and sequentially dehydrated in 35%, 50% and finally 70% ethanol in which they were stored.

With visualization through an operating microscope, the entire organ of Corti was dissected free from the osseous spiral lamina and each turn was mounted separately in glycerine. Using phase contrast microscopy, counts were made of the number of missing IHC's and OHC's in representative lengths of 100 cell segments at the base, turn 2, turn 3, turn 4 and at the apex of the cochlea. From these counts the percentage of missing OHC's and IHC's was easily computed for each location in each ear. The dissection and counting of the hair cells was conducted using a blind procedure.

Measurement of Arterial Blood Gas and pH:

In order to determine the direction and the magnitude of changes in arterial blood pH, PO_2 and PCO_2 resulting from four hours of carbogen inhalation in unrestrained unanesthetized guinea pigs seven additional animals were used. All blood samples were analyzed using a Radiometer-Copenhagen (PHM-27) blood gas analyzer. This instrument was calibrated before each series of blood measurements and corrected for barometric pressure daily.

The animals were first anesthetized by inhalation of Penthrane (Methoxyflurane) at room temperature and the trachea and carotid arteries exposed. A single carotid artery (right or left) was

dissected away from the neurovascular bundle and cannulated with a #22 teflon-coated intravenous catheter (Quik-Cath). The catheter was firmly tied to the vessel and sutured to the skin and fascia of the animal. The skin incision was closed with stainless steel clips and approximately 0.05 cc of sodium heparin was injected into the catheter to prevent blood clotting. All animals were then given 24 hours to recover from this procedure.

Following recovery approximately 0.75 cc of arterial blood was drawn into heparinized glass syringes with greased plungers which were stored on ice. Following the withdrawal of each sample, heparin was injected into the catheter. Five animals were placed into the exposure cage in a carbogen atmosphere and two animals placed in a normal air atmosphere. Conditions were identical to those in Experiment I with the exception that no experimental acoustic exposure was given. Samples of arterial blood were collected from these animals after 30, 60 and 360 minutes of exposure to gas (TABLE IV). One sample was collected from an animal after 30 minutes of recovery in room air.

All blood samples were stored on ice until measurement. No more than 3 hours was allowed to elapse between collection and analysis for any sample.

EXPERIMENT II - Effects of Carbogen Inhalation on Pure Tone Induced Temporary Depression of N_1 Evoked Potential Amplitude

Animal Selection

Ten healthy pigmented guinea pigs within a 200-300 gram weight

range were used in this study. The animals were randomly assigned into two experimental treatment groups depending on gas exposure. All animals in both groups received identical acoustic exposure consisting of a pure tone overload at 4.5 kHz presented for 600 seconds at 104 dB SPL. The groups differed only in that one respired carbogen gas for 20 minutes prior to and then 10 minutes during the acoustic overload while the other received room air during this time. Recovery of N_1 response amplitude was monitored for 3 hours after stimulation in all animals.

Surgical Preparation

Each animal was first anesthetized by an intraperitoneal injection of allobarbitol as previously described. Surgical preparation was also identical to that described in the previous section with the exception that in the present study the round window electrode was cemented to the bulla. This preparation allowed removal of the micromanipulator and relatively free movement of the animal without disturbing the contact between the round window membrane and the electrode. Rectal temperature was maintained at 38^{o} C $\pm$ 2^{o} C with a circulating warm water radiator constructed of polyethylene tubing.

The stroke volume of the respirator was adjusted to provide ample chest excursion on inhalation and then fine tuned to eliminate spontaneous middle ear muscle activity (MEMA)(68) as gauged by visual examination of the tympanic membrane. The respirator rate was consistently maintained at 60 breaths/minute.

For presentation of the acoustic test stimuli to the ear a sealed sound system was used. A specially designed sound cannula was sealed into the external auditory meatus with silicone grease. Care was taken to ensure a tight seal.

Acoustic Test Stimuli

Acoustic test stimuli were produced by a condenser microphone (Bruel and Kjaer, 1/2") driven in reverse in order to obtain as "flat" a sound field as possible. The microphone inserted into the top of the sound cannula with soft rubber fittings to ensure a tight seal. The distance from the diaphragm of the microphone to the tympanic membrane of the animal after placement was approximately 2.5 cm. The sound cannula also contained provision for a calibrated probe tube microphone (Bruel & Kjaer 1/2") with a 1 mm probe tube. The tip of the probe tube when in position was located approximately 7 mm from the tympanic membrane. The calibrated microphone, condenser microphone and sound cannula were left in place during acoustic exposures and all electrophysiological and acoustic measurements. Reproduction of the acoustic test stimuli was qualitatively assessed by examination of the AC cochlear potential which they elicited.

Electrical signals recorded from the round window membrane were lead to a battery powered differential amplifier (Grass P15-B) where they were filtered and amplified 100 times. The biological signals were further filtered and amplified by a plug-in amplifier (Tektronix #3A9) within the oscilloscope before being led to a

computer for averaging and storage. The oscilloscope also provided a useful visual monitor of the electrical signals.

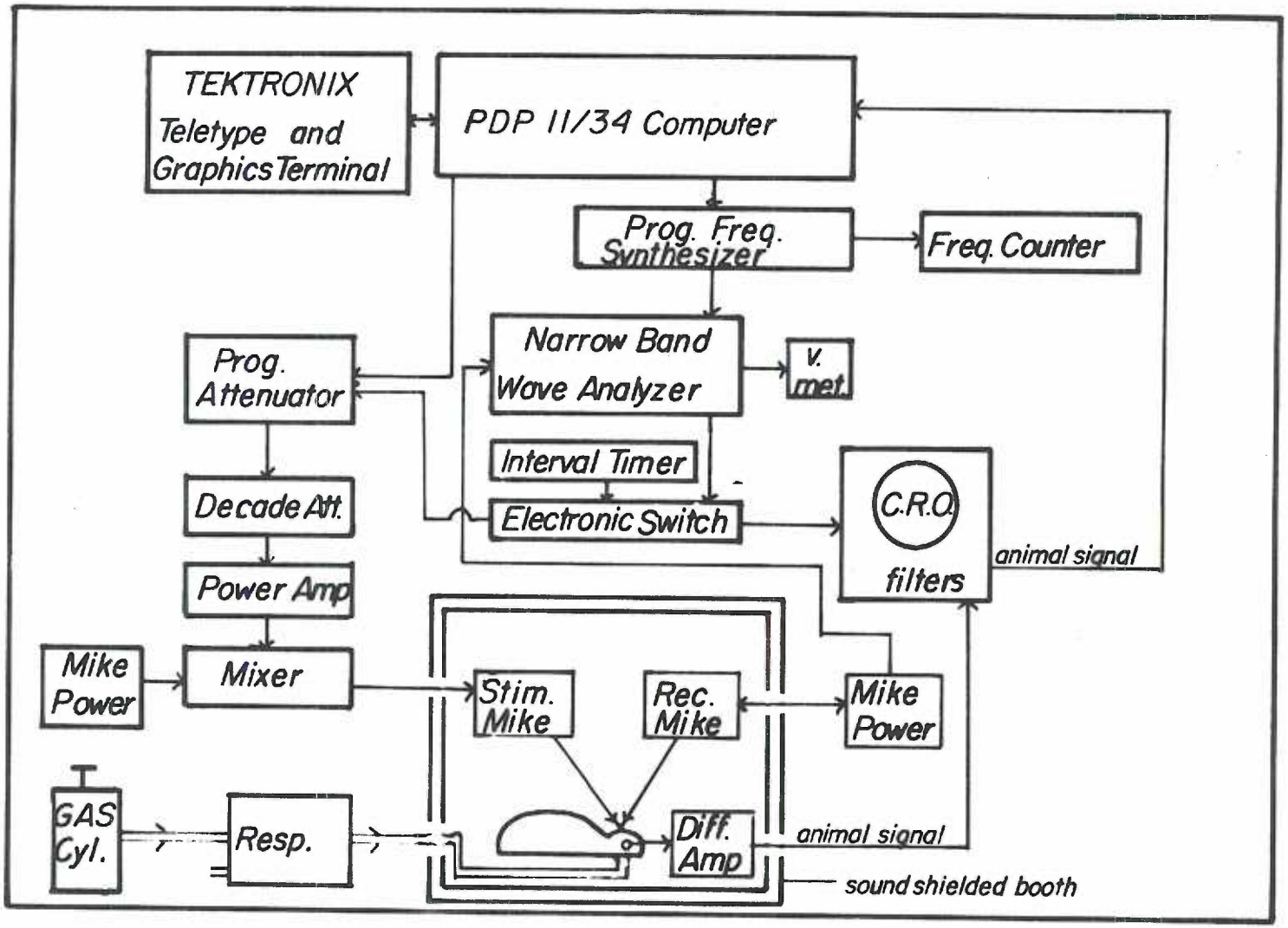
A PDP 11/34 computer was used to control the frequency and intensity of the acoustic test stimuli. The computer was also used for averaging and measurement of the N_1 responses. The experimental equipment and technique used for recording N_1 responses before and after overload was adapted from procedures developed by Meikle and Lee (69). A schematic diagram of the experimental set up is shown in Figure 4.

Frequencies were generated by a narrow band wave analyzer (General Radio 1162). The wave analyzer was also used during measurement of the intensities of the acoustic stimulus at each frequency.

Intensity of the acoustic stimuli was controlled at two points. One was by a programmable dB attenuator (Coulbourn S85-08) under computer control and the other by a decade attenuator under the experimenters manual control. The latter attenuator was positioned to provide tail-end attenuation of noise produced by the Coulbourn attenuator. For this purpose the decade attenuator was set at 20 dB during all data collection. The degree of attenuation present in the programmable attenuator varied with each stimulus frequency. During the acoustic overload the decade attenuator was set at 10 dB with 0 dB present in the programmable attenuator. Final amplification of the acoustic stimuli was provided by a Mackintosh 105 watt power amplifier.

Acoustic test stimuli consisted of shaped tone bursts having a

Figure 4. Schematic diagram of the sound producing system, sound measurement system and the N_1 response recording system used in Experiment II. The active, reference and recording electrodes are not labeled. Note that the animal preparation and bioamplifier are enclosed within an electrically isolated sound shielded chamber. Note placement of decade attenuator with the purpose of attenuation of noise produced by programmable attenuator.


TEKTRONIX
Teletype and
Graphics Terminal
PDP 11/34 Computer
Prog. Freq.
Synthesizer
Freq. Counter
Prog.
Attenuator
Narrow Band
Wave Analyzer
V.
met.
Interval Timer
Decade Att.
Electronic Switch
C.R.O.
filters
animal signal
Power Amp
Mike
Power
Mixer
Stim.
Mike
Rec.
Mike
Mike
Power
GAS
Cyl.
Resp.
Diff.
Amp
animal signal
sound shielded booth

10 msec duration, a 1 msec rise and decay time and a 100 msec inter-stimulus interval (14) (76). Phase of tone burst onset was random. Timing and gating of the tone bursts was accomplished using an interval timer (Grason-Stadler 471-1) and an electronic switch (Grason Stadler 829-E). Tone bursts were presented at 32 frequencies from 2100 Hz through 30 kHz. (APPENDIX B). For convenience, these frequencies were numbered by integers 1 through 32, each frequency being separated from the next by 1/8 of an octave. The N_1 responses were sampled by the computer using a 12 bit A/D converter with a sampling rate of 25 kHz.

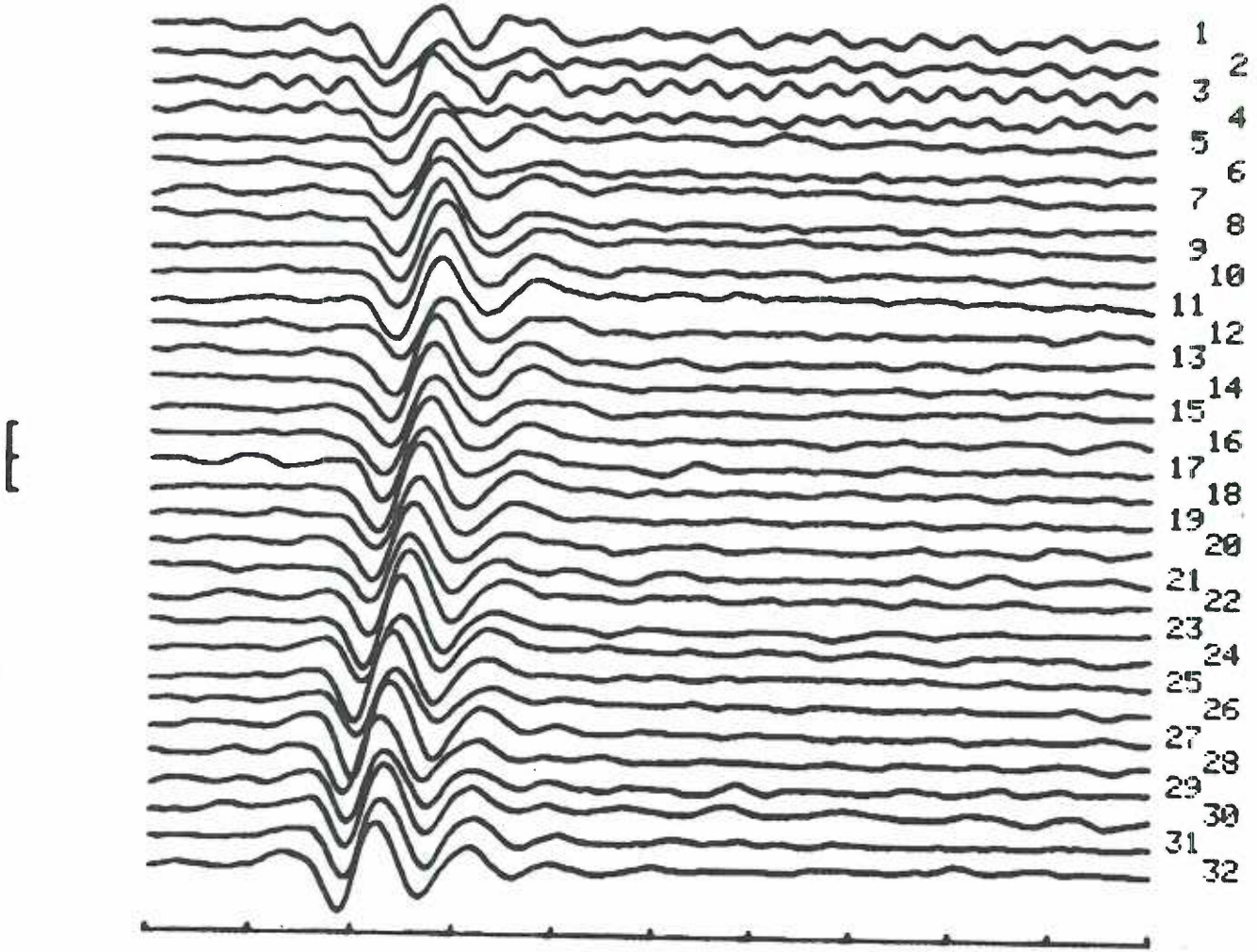
Recording of N_1 Responses

The sound intensity of the tone bursts at each frequency was adjusted in order to produce N_1 responses of 50 μV at that frequency. The attenuation setting required at each of the 32 frequencies was stored in the computer memory. This list of intensities was used during each N_1 test sequence.

During a Test Sequence (TS) a total of 10 stimulus repetitions at each frequency was presented in order to compute an averaged N_1 response. A complete TS consisted of 320 tone burst presentations. The order of presentation of each tone burst was pseudo-random with the constraint that no two consecutive tone bursts were closer together than 2 octaves. This constraint served to minimize N_1 response adaptation. The time required to complete a TS was approximately 32 seconds (32 frequencies x 10 trials x 100 msec). Each complete TS provided an array of 32 averaged N_1 responses (Figure 5)

Figure 5. A sample array of N_1 responses at 32 frequencies from 2.1-30 kHz taken approximately 2000 seconds before initiation of the acoustic overload (TS-2). Note that the intensity of the acoustic stimulus at each frequency has been adjusted to provide an averaged N_1 response of 50 μv.

SAMPLE TS N1 RECOVERY G P #10(CARBOGEN), TIME POST-EXP= TS 2 -1977 SEC



which was stored in memory for off-line measurement.

Experimental Procedure

Immediately following adjustment of the programmable attenuator to yield 50 μV N_1 responses at each test frequency, two TS's were performed. These were displayed for qualitative examination of response reliability. Display of frequencies 8-22 followed every subsequent TS to provide an on-line monitor of N_1 amplitude. Hard copies of each TS display were made at the time of the TS. Following completion of TS-2, a carefully timed experimental procedure was begun.

At this time the intake of the respirator was either connected to a small cylinder of carbogen gas (Carbogen Group) or left in communication with room air (Room Air Group). The carbogen was delivered through a reservoir bag at a flow rate of 4.2 liters/minute. Both room air and carbogen were humidified before delivery to the animal to control tracheobronchial dehydration.

Respiration of carbogen or of room air continued for exactly 20 minutes whereupon TS-3 was conducted. This allowed inspection of the N_1 responses to determine whether there were any changes resulting from the gas exposure or the 20 minute interval. TS-1, TS-2 and TS-3 were averaged together during off-line N_1 amplitude measurement to provide the baseline responses at each frequency for each ear. Immediately following TS-3, the acoustic overload was begun.

Acoustic Overload

The acoustic overload consisted of a 4.5 kHz pure tone (Frequency 10) presented at an intensity of 104 dB for 600 seconds. Duration of acoustic exposure was under computer control. Intensity of the acoustic stimulus was adjusted before TS-3 for each ear using the following method.

With 0 dB in the programmable attenuator and 40 dB in the decade attenuator the gain of the power amplifier was increased to provide exactly 1 µbar at 4.5 kHz within the sound cannula (1 µbar = 74 dB re: 20 µPa) measured by the probe tube microphone. The amount of attenuation in the decade attenuator was decreased 30 dB during the acoustic overload to correspond to an actual SPL of 104 dB.

At the end of the 600 second acoustic overload the carbogen (if used) was disconnected from the respirator intake and all animals respired room air for the remainder of the experiment. It was estimated that no more than 2 minutes were required for complete flushing of carbogen from the respirator and respirator tubing. Apart from this transition period, the animals in the carbogen group received a total of 30 minutes of gas exposure (20 minutes prior to and 10 minutes during acoustic overload). Timing of gas exposure duration and initiation of all test sequences was accomplished manually using a digital stopwatch.

Prior to the last TS (TS-15) the sound pressure at each of the 32 test frequencies was measured. From these measures and the intensities required at each frequency to produce 50 µV N1 responses,

a 50 μV isopotential function of the N_1 could be constructed for each ear.

Recovery of N_1 Response Amplitude

Test sequences were conducted at 12 precisely timed points following the acoustic overload (Figure 6). The points corresponded to 0.33, 2, 5, 10, 15, 20, 30, 40, 60, 90, 120 and 180 minutes post exposure.

The peak-to-peak amplitude of each averaged N_1 response from each of these TS's (32 responses x 15 TS's = 480 responses/animal) was measured using an automated computer measurement technique.* To minimize contamination of the N_1 responses by AC cochlear potentials, all N_1 traces were digitally filtered using a 2-pole Butterworth high pass filter function (93) with a corner frequency of 1.5 kHz. The filtering process introduced approximately 2-3 dB of attenuation into each N_1 trace as well as a slight phase shift. The bias introduced by the filtering procedure was not considered to significantly affect the conclusions to be drawn from the present study.

From the N_1 amplitude measures, plots of N_1 response depression and recovery over time at any frequency could be constructed as well as plots of depression at each frequency within each TS.

Arterial Blood Gas Measures

In order to determine the approximate changes in arterial pH,

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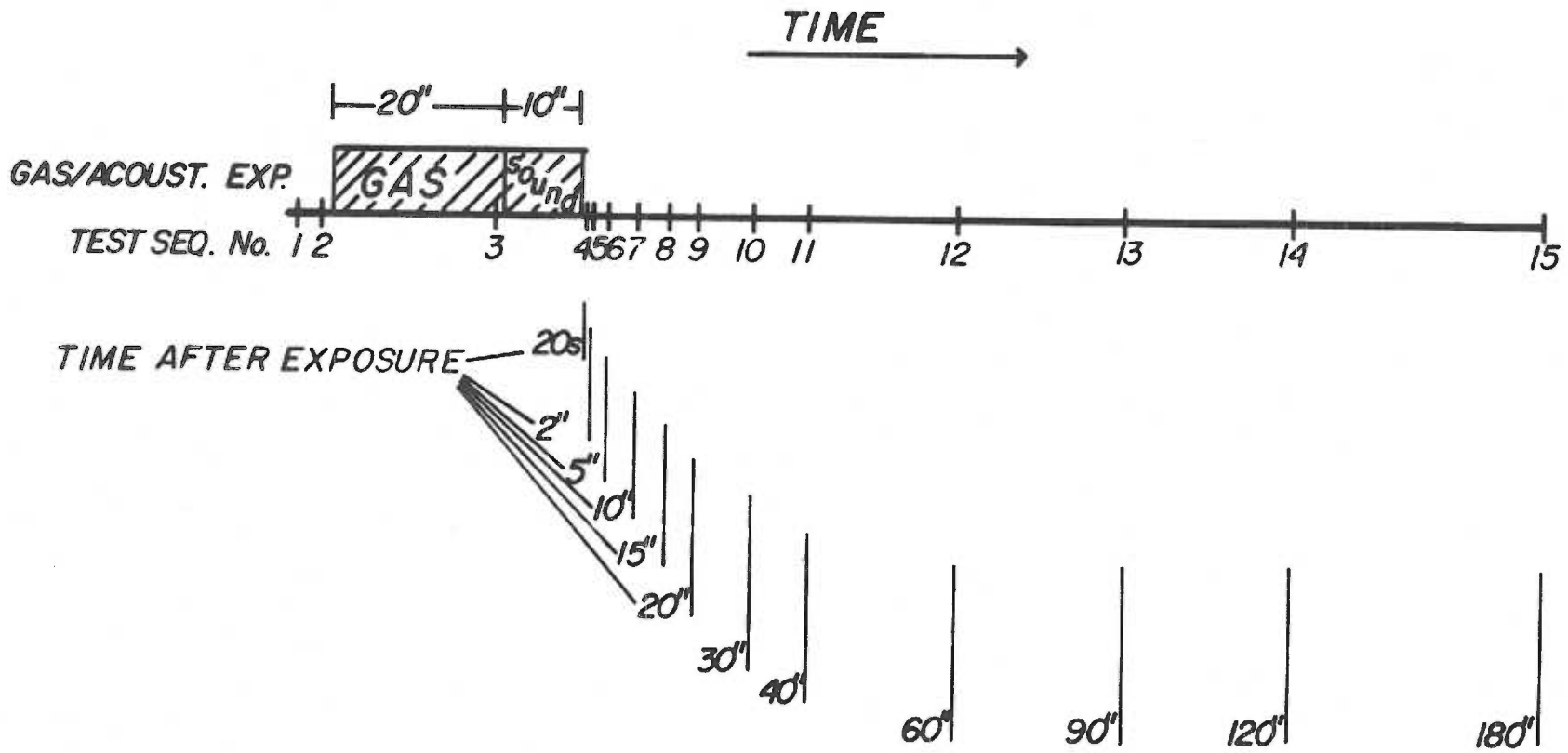
*The author wishes to thank Mr. Craig Lee for developing and modifying the computer programs for this study.

Figure 6. Schematic time-line demonstrating the sequence and timing of events in Experiment II. Note that two Test Sequences were performed before the experimental gas was introduced (Carbogen or Room Air) and one performed during gas exposure (TS-3). Note also that gas exposure continued throughout the 10 minute, 4.5 kHz, 104 dB pure tone acoustic overload. Timing of Test Sequences following cessation of the acoustic overload is indicated next to the vertical bars at the bottom of the figure.


TIME
20"
10"
GAS/ACOUST. EXP.
GAS
sound
TEST SEQ. No. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME AFTER EXPOSURE
20s
2"
5"
10"
15"
20"
30"
40"
60"
90"
120"
180"

PO_2 and PCO_2 induced by the carbogen inhalation in this experiment four additional guinea pigs were prepared as follows. Animals were anesthetized by intraperitoneal injection of allobarbitol and placed on artificial ventilation as previously described. The ventilator volume was adjusted to provide approximately the same degree of chest excursion as observed in the preceeding series of 10 animals. The left or right carotid artery was cannulated and the cannula tied into place. Heparin was instilled into the distal end of the cannula. Samples of arterial blood were drawn into heparinized glass syringes with greased plungers at various times before, during and after exposure to either carbogen gas or room air. Syringes were stored on ice until measures could be made as previously described.

Analysis of Data, Experiment I

All animals survived the experimental procedure and the 30-40 day stabilization period. Acceptable electrophysiological measures were obtained from all ears in each group of animals. Because the electrophysiological and the histological measures from the two Control Groups were identical over all test frequencies and locations within the cochlea, these two groups of 5 animals were pooled for statistical analysis. Some fixation artifact was created at the apex of some of the cochleae during fixative perfusion. If it was possible, counts were made of missing hair cells from intact segments at this site, otherwise counts of missing inner or outer hair cells were not included in the statistical analysis.

Statistical analysis of all raw data was performed using each ear of each animal as an independent subject. This provided an N of 20 ears in each of the three experimental groups. Analysis was performed using the analysis of variance and the Scheffé test which measures group by group variations (17). Computations were made on a Wang Model 720 minicomputer. The analysis of variance of the 1 μV isopotential functions involved 3 one way ANOVA's using a 1 by 3 design. These corresponded to the elevation of the isopotential function (a) over-all test frequencies; (b) over frequencies from 100 Hz through 1.5 kHz; and (c) over frequencies from 2 kHz through 30 kHz. Analysis of intensity functions of the AC cochlear potential was accomplished using the maximum electrical output that each ear was able to attain at 1 kHz and at 10 kHz in two 1 by 3 designs. Analysis of IHC and OHC counts were performed using a 2 way ANOVA in a 3 by 5 design with repeated measures on location within the cochlea. The Scheffé test followed the analysis of variance in each case.

Analysis of Data, Experiment II

In pilot experimentation performed in preparation for Experiment II it was observed that a large amount of variability existed from animal to animal with regard to sensitivity to the acoustic exposure. In order to make meaningful comparisons of animals between the two experimental groups it was necessary to design a procedure by which animals could be matched for auditory sensitivity and then each matched pair of animals compared for amount of cochlear

impairment resulting from the acoustic overload. Animals in the Carbogen Group were matched with animals in the Room Air Group in the following manner. From the measures of the amount of sound pressure at each frequency in each ear and the amount of sound required to produce a 50 μV N_1 response in that ear at each frequency, 50 μV isopotential functions of the N_1 potential could be constructed for each ear (Figure 7). From these isopotential functions a mean of the values within the range of frequencies presumed to be most affected by the acoustic stimulus was computed (frequencies 9-18).

The number thus computed was taken to represent a measure of that ear's auditory function. The value for each ear was then rank ordered in each group to determine which animal should be matched with another (TABLE II). A difference level of $\pm$ 3 dB was a priori considered to indicate similar levels of cochlear sensitivity and therefore a good match.

Each of the matches represented in TABLE II fell within the acceptable range with the mean difference score amounting to 3 dB. Because each of the animals in each group was matched with one in the other group and because the mean difference score was low, the data from all animals in each group was pooled for statistical analysis.

Pooling of the data was accomplished by transformation of the raw N_1 response amplitudes from its value in μV (peak-to-peak) to a percentage value relative to the mean N_1 amplitude measured before acoustic exposure in TS-1 - TS-3 (APPENDIX I). The percentage

Figure 7. 50 μv isopotential function of the N_1 response at frequencies from 2.1 kHz through 30 kHz from two matched animals in Experiment II. G.P. #1 and G.P. #4 are shown. Horizontal bar at the top of the figure demonstrates the range of frequencies averaged for the purpose of matching animals from each experimental group (TABLE II).

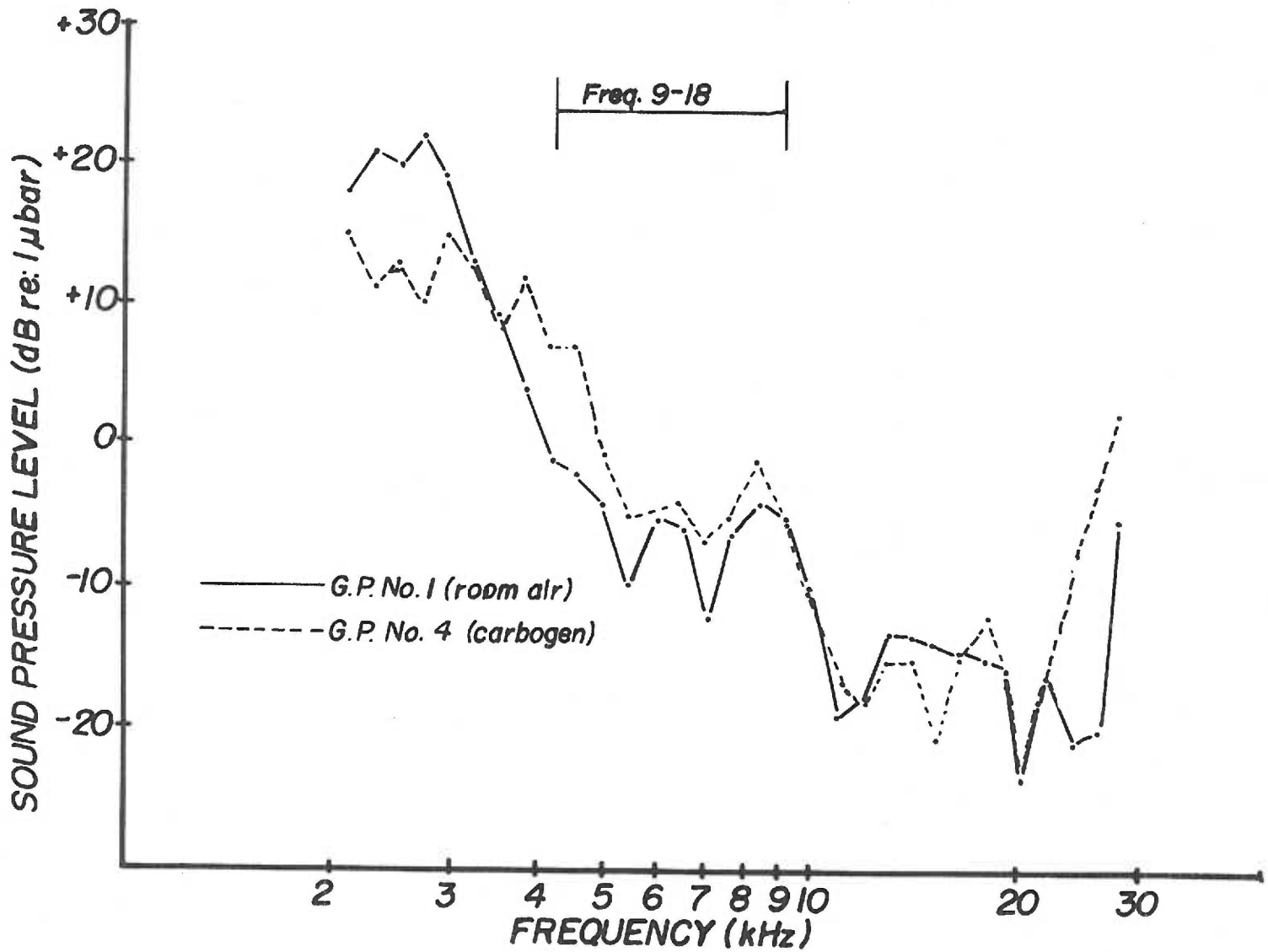

+30
+20
+10
0
-10
-20
SOUND PRESSURE LEVEL (dB re: 1 µbar)
Freq. 9-18
G.P. No. 1 (room air)
G.P. No. 4 (carbogen)
2
3
4
5
6
7
8
9
10
20
30
FREQUENCY (kHz)

TABLE II. Listing of mean sound pressure required to produce 50 μv peak to peak N_1 responses at frequencies 9 through 18 (4.2-9.1 kHz) obtained from ten guinea pigs. Values are rank ordered from the most to the least sensitive ear in each of two experimental groups. This rank order has been used as a basis for comparison of individual animal measures between the two groups. The overall analysis of data from Experiment II was accomplished using group means. The difference score represents the approximate correspondence in sensitivity from ear to ear.

TABLE II

Room Air Group		-		Carbogen Group
Animal	Mean dB	Difference (dB)	Mean dB	Animal
G.P. #3	-10.3	5.8	-16.1	G.P. #6
G.P. #13	-5.1	0.0	-5.1	G.P. #10
G.P. #1	-4.5	2.5	-2.0	G.P. #4*
G.P. #8	+1.4	2.8	-1.4	G.P. #9
G.P. #12	+4.4	4.2	+0.2	G.P. #2
Mean Values:	-2.8	3.0	-4.8	

* Representative pair of animals used for data display.

values in TS-4 through TS-15 were then averaged within each TS for several sets and subsets of frequencies. Mean relative depression (RD) curves were constructed for all frequencies in each TS following acoustic overload. Mean relative recovery (RR) curves were constructed for frequencies number 1-32, number 1-12, number 19-32, number 13-18 and number 14-16 inclusive. These values were further analyzed using the analysis of variance (17). Analysis of RR curves from each set of frequencies was accomplished using a 2-way ANOVA involving a 2 by 12 design with repeated measures on B, Test Sequence. The Scheffé test followed the analysis of variance in each case.

Plots of RD curves and RR curves were also constructed for one pair of matched animals (G.P.#1 and G.P. #4). It may be seen in TABLE I that this pair of animals represent a median match in the rank order. It may be seen in Figure 7 that the 50 μV isopotential functions of the N_1 response for these two animals are very similar. Whenever raw data is presented in the following test it is from these two animals.

RESULTS

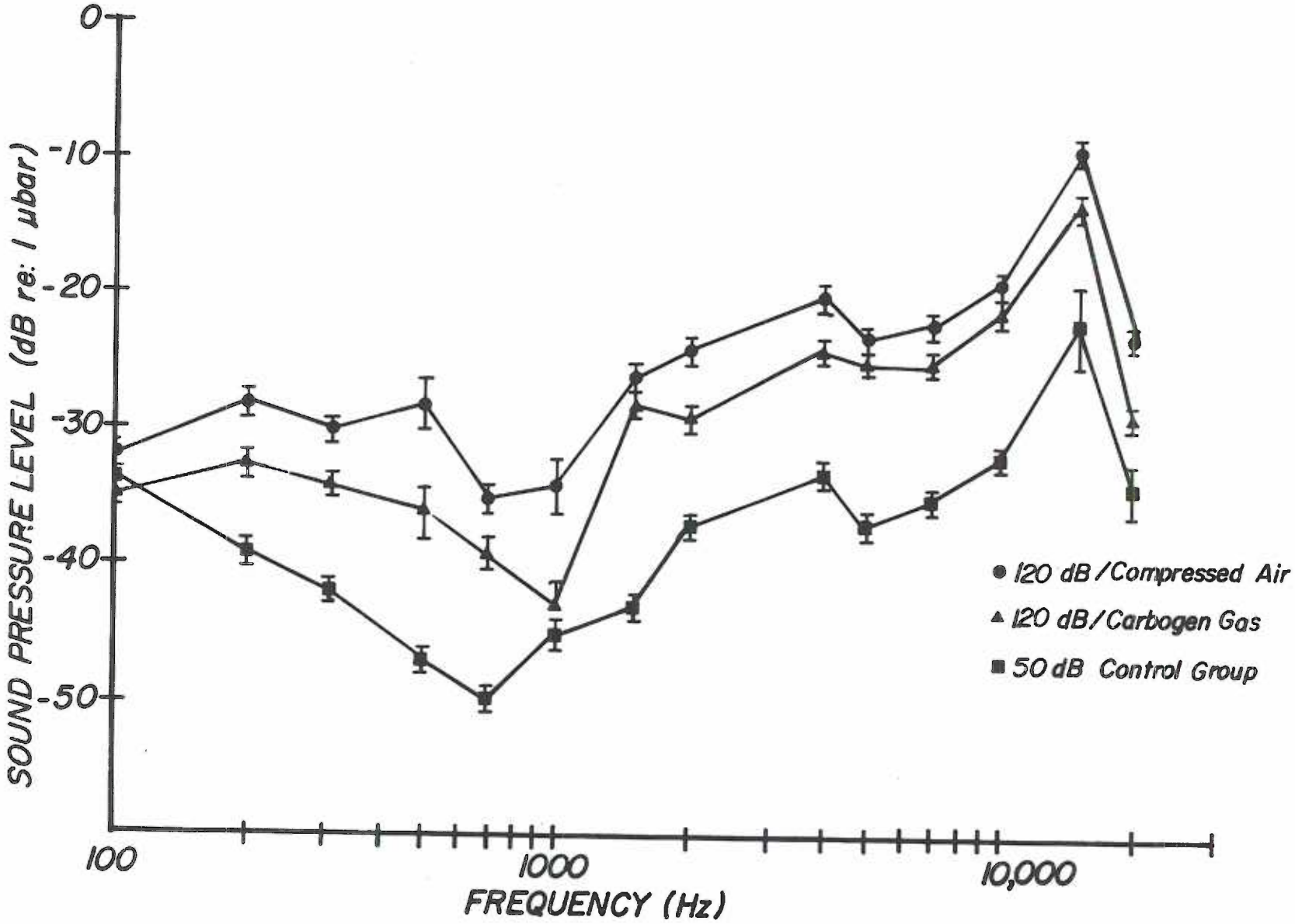
The results of both experiments confirmed a beneficial effect of carbogen inhalation on noise-induced auditory impairment. In Experiment I a decrease in noise-induced permanent elevation of the 1 μV isopotential function and in the number of missing inner and outer hair cells was found in animals given carbogen during acoustic exposure compared to those given compressed normal air. In Experiment II a decrease in the degree of depression, especially in the early stages of recovery, from a pure tone acoustic overload was found in animals given carbogen during exposure compared to those given room air. Within all measures of cochlear integrity, with the exception of the maximal outputs of intensity functions in Experiment I, the inhalation of carbogen during acoustic exposure introduced a statistically significant shift in all measures in the direction of increased auditory function.

EXPERIMENT I

Electrophysiological Measures

The mean 1 μV isopotential functions of the AC cochlear potential for each of the 3 experimental groups within a frequency range of 100 Hz through 20 kHz are shown in Figure 8. Each point on each curve represents the mean sound level required at that frequency to elicit a cochlear potential of 1μV (RMS). The sound pressure level required to attain this electrical output is plotted

Figure 8. Averaged 1 μv isopential functions of the AC cochlear potential obtained from all ears of 3 groups of animals in Experiment I. Vertical bars represent ± 1 standard error of the mean (SEM). Major auditory impairment was identified in the group which received 120 dB of broad band noise in an atmospere of normal air (120 dB/Compressed Air) shown at the top of the figure. Exposure to identical acoustic overexposure in an atmosphere of Carbogen gas (120 dB/Carbogen Gas) led to less severe auditory impairment. The Control group curve may be seen at the bottom of the figure.


0
-10
-20
-30
-40
-50
SOUND PRESSURE LEVEL (dB re: 1 µbar)
100
1000
10,000
FREQUENCY (Hz)
● 120 dB/Compressed Air
▲ 120 dB/Carbogen Gas
■ 50 dB Control Group

on the ordinate and is inversely proportional to the functional performance of the ear. Thus the extent of cochlear damage is represented by the relative height of each curve above that of the control group.

The Compressed Air/120 Group is represented by the uppermost curve in Figure 8. These animals received 17.5 hours of broad band noise at 120 dB while breathing compressed air. Showing the effects of the acoustic overstimulation, but to a lesser degree, is the Carbogen/120 Group curve in the middle of Figure 8. These animals were exposed to an identical level of acoustic exposure while breathing carbogen gas. The Control Group curve at the bottom of Figure 8 provides a measure of the performance of normal ears.

It is evident from visual inspection of Figure 8 that the inhalation of carbogen during acoustic exposure led to a decrease in the pathologic elevation of the 1 μV isopotential function at each test frequency. When averaged across all test frequencies, the elevation of the Compressed Air/120 Group curve amounted to 13 dB relative to the Control Group. In contrast, the mean elevation of the Carbogen/120 Group curve was only 8 dB. The decrease in elevation of the isopotential function in the group which received carbogen was most pronounced at frequencies below 1.5 kHz.

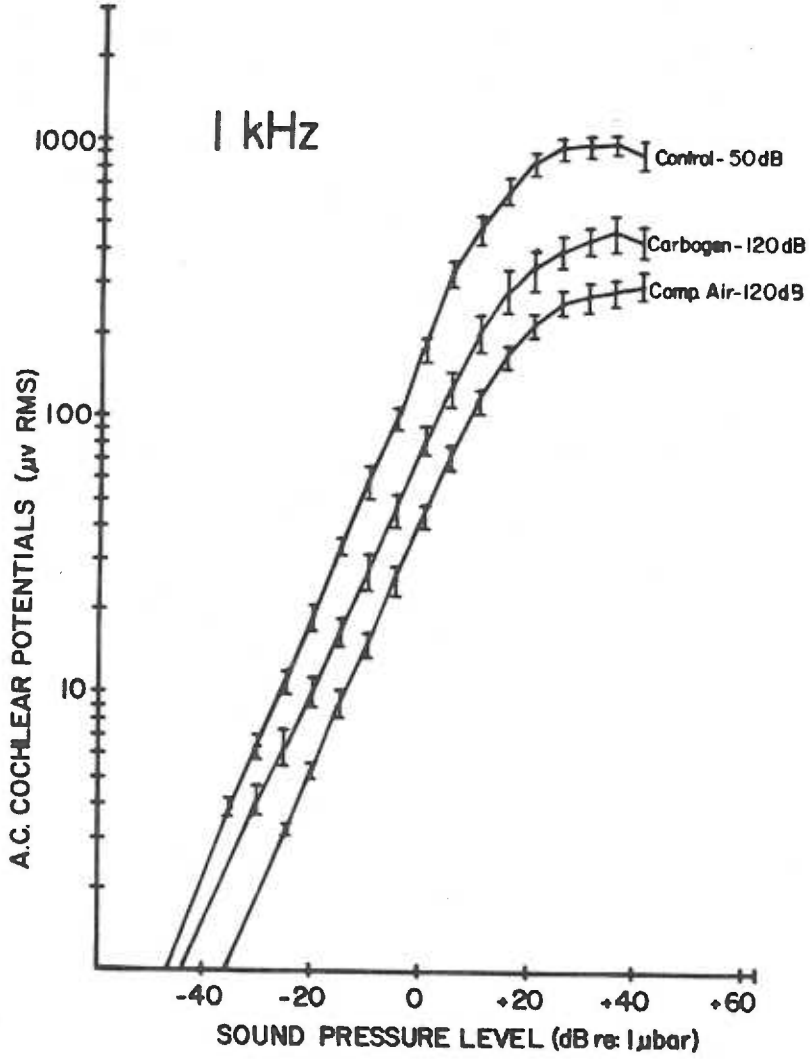
The analysis of variance applied to the raw data from the 1 μV isopotential functions demonstrated a significant effect due to treatment ($F_{(2,57)} = 64.9$, $p < .001$). The Scheffé test applied to these means indicated that the Carbogen/120 and the Compressed Air/120 Groups differed at the $p < .005$ level of significance. Both

of the high-intensity acoustically-exposed groups differed from the Control Group ($p < .001$). At frequencies below 1.5 kHz the difference between the Carbogen/120 and the Compressed Air/120 Groups was more pronounced ($p < .001$), whereas at frequencies above 1.5 kHz the difference was less pronounced ($p < .05$) (APPENDIX C). Variability within the three experimental groups was approximately equivalent.

The AC cochlear potential output in μV (RMS) resulting from a sound stimulus of increasing intensity at 1 kHz and at 10 kHz are shown in Figure 9. It is evident from inspection of these intensity functions that inhalation of carbogen gas during acoustic overexposure resulted in a shift of the curves to the left and upwards relative to those animals which received only normal air during acoustic overexposure. The direction of this shift indicates an increase in sensitivity as well as in the output capability of the ears. The mean maximal outputs of the AC cochlear potential at 1 kHz and at 10 kHz are shown in TABLE III.

The analysis of variance applied to these data demonstrated significant difference between the means of the acoustically exposed groups (120 dB) and the Control Group (50 dB) but not between the Carbogen/120 and the Compressed Air/120 Groups (APPENDIX D) either at 1 kHz or at 10 kHz. However, no significant difference was found at 10 kHz between the maximal output of the Carbogen/120 Group and the Control Group while the Compressed Air/120 Group did differ from the Controls ($p < .05$).

Figure 9. Intensity functions of the AC cochlear potential at 1 kHz and at 10 kHz obtained from 3 groups of guinea pigs. Each curve represents the mean of all ears within the appropriate treatment group at each point. Vertical bars represent ± 1 SEM.


1 kHz
1000
100
10
A.C. COCHLEAR POTENTIALS (μv RMS)
Control - 50 dB
Carbogen - 120 dB
Comp. Air - 120 dB
-40
-20
0
+20
+40
+60
SOUND PRESSURE LEVEL (dB re: 1 μbar)

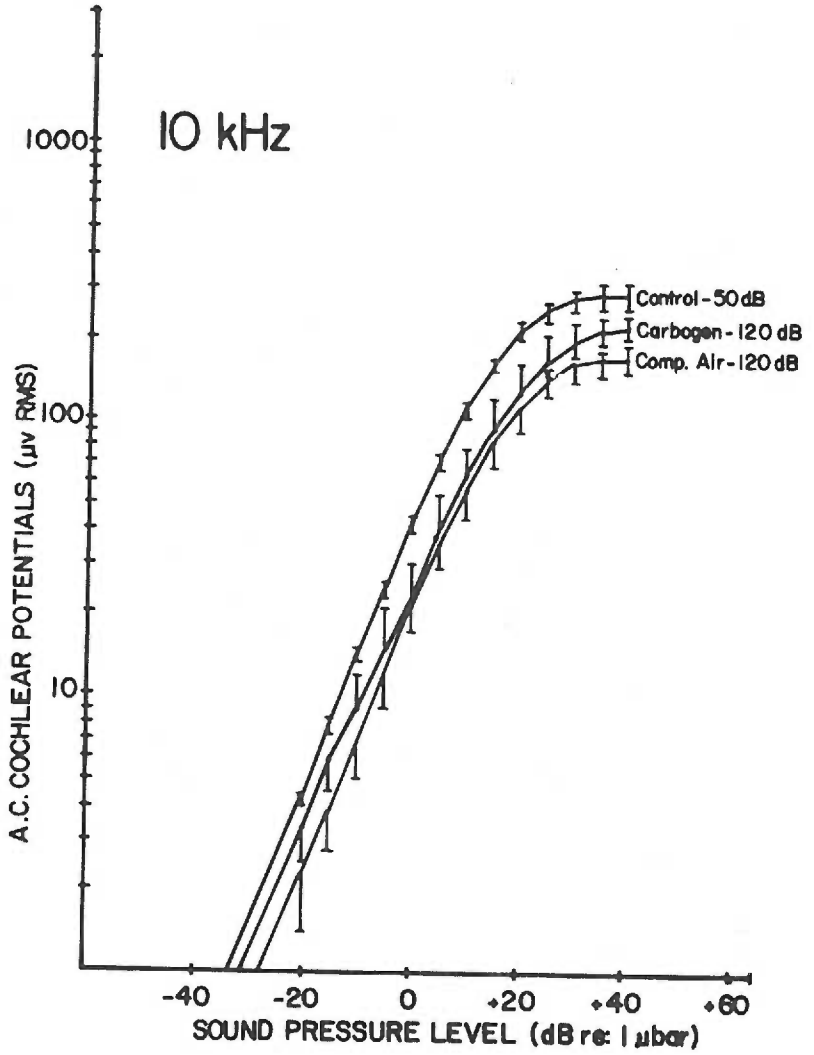

10 kHz
1000
100
10
A.C. COCHLEAR POTENTIALS (μv RMS)
Control - 50 dB
Carbogen - 120 dB
Comp. Air - 120 dB
-40
-20
0
+20
+40
+60
SOUND PRESSURE LEVEL (dB re: 1 μbar)

TABLE III. Maximum electrical output of the AC cochlear potential at 1 kHz and at 10 kHz obtained from 3 groups of animals. Values are the mean of all ears within each treatment group and are reported in RMS voltage.

TABLE III

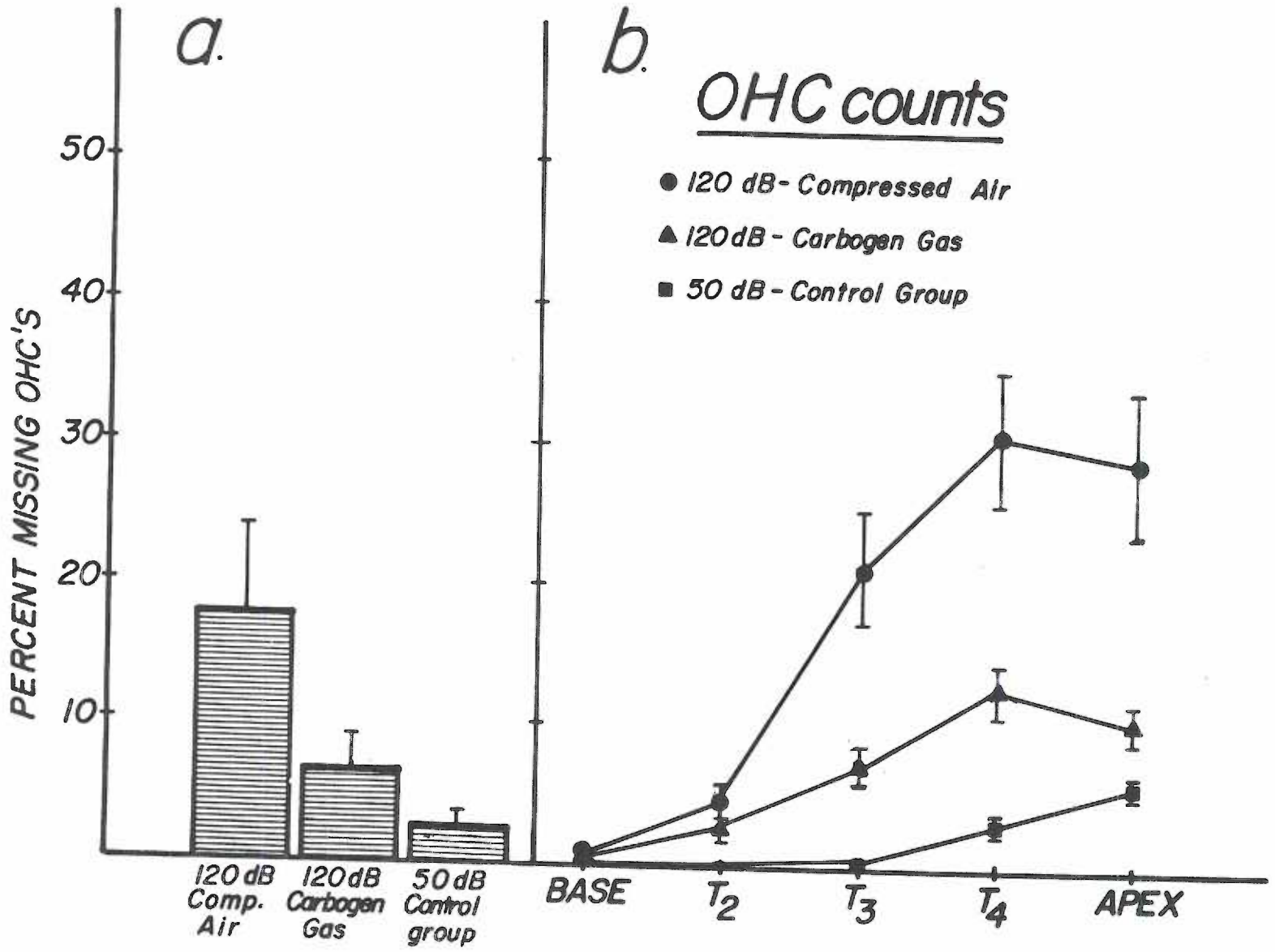
Experimental Treatment Group	Maximum Output at 1 kHz (RMS)	Maximum Output at 10 kHz (RMS)
Control	1010 μv	282 μv
Carbogen/120	434 μv	263 μv
Compressed Air/120	361 μv	207 μv

Histological Findings

Cochlear pathology in the groups exposed to 120 dB noise was characteristic of the type of hair cell loss resulting from a metabolic lesion rather than from pure mechanical trauma. OHC loss was patchy in both groups with common findings of only one or two OHC's lost within a single row. In ears which showed a larger degree of OHC loss it was common to find scattered groups of 2-3 OHC's to be missing from each row of OHC's at a single site. IHC loss was morphologically similar to OHC loss. Again scattered groups of inner hair cells were found to be missing rather than continuous bands along the basilar membrane. In general, on the basis of cochlear morphology, the use of 17.5 hours of 120 dB broad band noise was confirmed as a level of acoustic exposure which provided a maximum of metabolic stress of the hair cells (see introduction) with a minimum of mechanical trauma.

Total OHC losses expressed as percentage values relative to the total number that would exist in an equivalent segment of perfect organ of Corti are shown in Figure 10a. These total OHC losses are broken down by location within the cochlea in Figure 10b. Complete listing of individual cochlear OHC losses is available in APPENDIX E. Total OHC loss averaged over all locations within the cochlea in the Control Group amounted to 2%, a figure that is commonly found in untreated guinea pigs. In the Compressed Air/120 Group the net loss of OHC's throughout the cochlea amounted to 17.4%. In contrast, the net loss of OHC's in the Carbogen/120 Group was found to be 6.5%. The analysis of variance applied to these data

Figure 10. Results of outer hair cell counts expressed as percentage values relative to the total number of OHC's that would exist in an equivalent segment of undamaged organ of Corti. Counts averaged over all areas of the cochlea are shown in (a) for each of 3 experimental treatment groups. These counts are shown as a function of location within the cochlea in (b). Vertical bars represent ± 1 SEM.

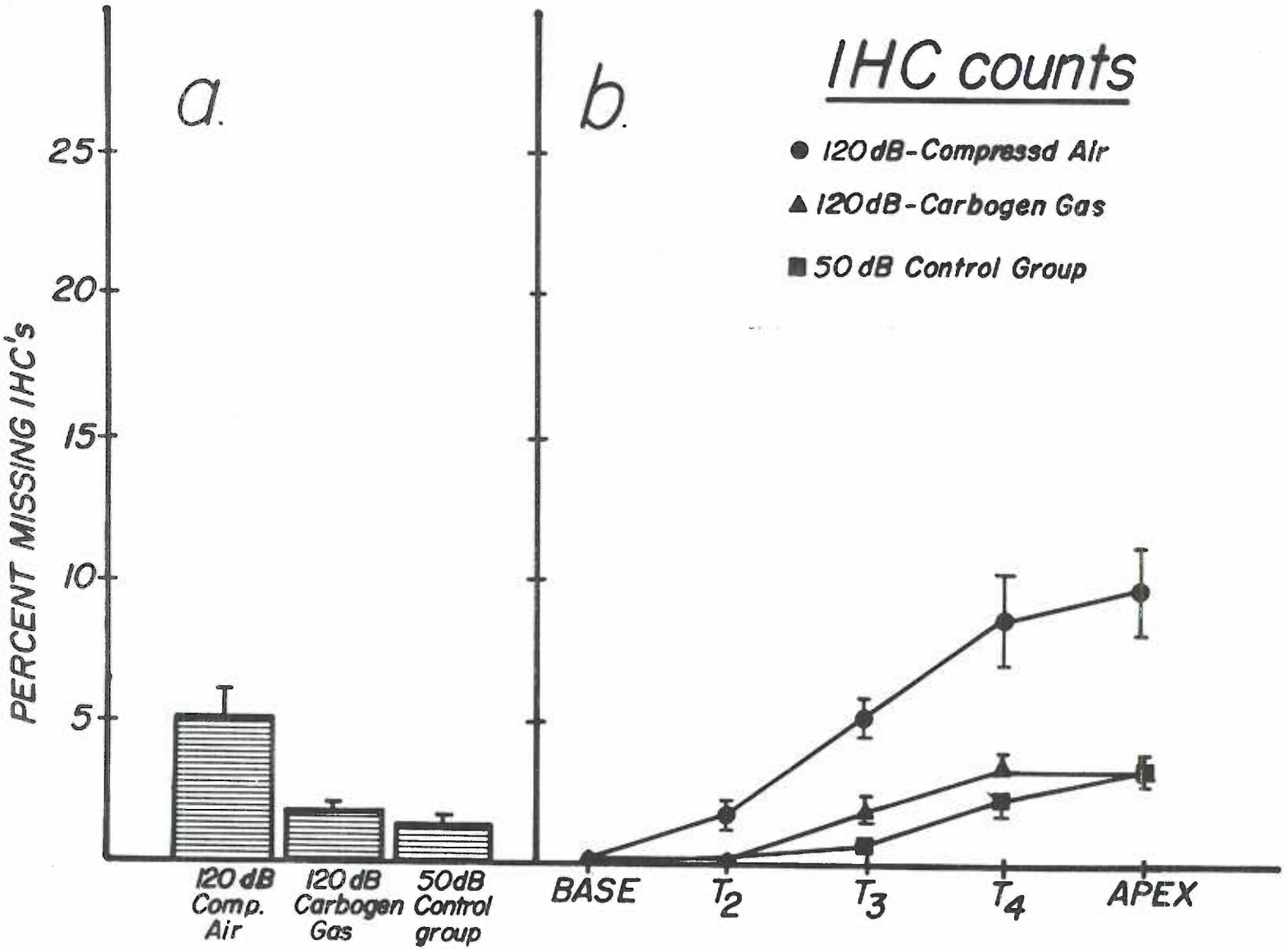

a.
b.
OHC counts
120 dB - Compressed Air
120dB - Carbogen Gas
50 dB - Control Group
PERCENT MISSING OHC'S
50
40
30
20
10
120 dB Comp. Air
120dB Carbogen Gas
50 dB Control group
BASE
T2
T3
T4
APEX

demonstrated a significant effect due to acoustic exposure ($F_{(2,57)} = 43.5$, $p < .001$) (APPENDIX F). The Scheffé test applied to the means revealed a significant difference between the Carbogen/120 and the Compressed Air/120 Group ($p < .001$).

It is evident from visual inspection of Figure 10b that the majority of OHC damage due to acoustic overload occurred within Turn 3 and Turn 4 of the cochlea. This area corresponds to a segment of basilar membrane approximately 0.4 to 6.19 mm from the apex (26) and to the frequencies 24 Hz - 1258 Hz according to Greenwood (34). This finding is consistent with the finding of a larger difference between means of the 1 μV isopotential function at these frequencies between the Compressed Air/120 and the Carbogen/120 Groups. By the Scheffé test the means of the OHC loss in these two groups were found to differ significantly at the apex ($p < .001$) at Turn 4 ($p < .001$) and at Turn 3 ($p < .001$) but not within Turn 2 or the base of the cochlea. The OHC loss in the Control Group was primarily found at the apex.

Total IHC losses expressed as percentage values relative to the total number of IHC that would exist in an equivalent segment of perfect organ of Corti are shown for each individual cochlea in APPENDIX G and graphically in Figure 11a. These IHC losses are broken down by location in the cochlea in Figure 11b. Total IHC losses averaged over all locations in the cochlea amounted to 1.2% in the Control Group, 4.5% in the Compressed Air/120 Group and 1.66% in the Carbogen/120 Group. The analysis of variance applied to these data demonstrated a significant effect due to acoustical exposure only in the Compressed Air/120 Group ($F(2,57)=19.78$, $p<.001$)

Figure 11. Results of inner hair cell counts expressed as percentage values relative to the total number of IHC's that would exist in an equivalent segment of undamaged organ of Corti. Counts averaged over all areas of the cochlea are shown in (a) for each of 3 experimental treatment groups. These counts are shown as a function of location within the cochlea in (b). Vertical bars represent ± 1 SEM.


a.
b.
IHC counts
120 dB-Compressd Air
120 dB-Carbogen Gas
50 dB Control Group
PERCENT MISSING IHC's
25
20
15
10
5
120 dB Comp. Air
120 dB Carbogen Gas
50 dB Control group
BASE
T2
T3
T4
APEX

(APPENDIX H).

Inspection of Figure 11b reveals striking similarity to the OHC losses in Figure 10b. Again, the majority of hair cell damage was above Turn 3 in all groups. The means of IHC loss in the Compressed Air/120 and Carbogen/120 Groups were found to differ significantly at the apex ($p < .001$) at Turn 4 ($p < .001$) and at Turn 3 ($p < .001$) but less so at Turn 2 ($p < .05$) and not at all in the base. Again, missing IHC's in the Control Group were identified toward the apex.

It should be pointed out that a fairly consistent ratio between OHC and IHC loss was found in the two high-intensity acoustically-exposed groups. In all cases, OHC's showed relatively more pathology then IHC's resulting from the same acoustic exposure, that is ratio % missing OHC's/% missing IHC's was > 1.

Arterial Blood Gas Measures

The arterial pH, PO_2 and PCO_2 obtained from the carotid artery of seven guinea pigs exposed to identical gas conditions as those in the preceeding experiment are shown in TABLE IV. The average arterial pH before placement into the exposure cage was 7.38. After 30 minutes of respiration of carbogen gas this dropped to 7.28. After 60 minutes arterial pH was found to be 7.31. After 4 hours it was once again found to be approximately 7.26. After 30 minutes of recovery in room air the pH in one animal was found to be 7.5. Arterial PCO_2 showed a corresponding shift. Before placement it averaged approximately 24 mm Hg which climbed to 36 mm Hz after

TABLE IV. Arterial blood pH, PO_2 and PCO_2 obtained from the carotid artery of seven guinea pigs. Five animals received 4 hours of exposure to carbogen gas and two animal received exposure to normal air. All animals were awake, unrestrained and unanesthetized for 24 hours prior to sampling. Animals which received carbogen gas are listed in IV-A, those that received normal air are listed in IV-B.

TABLE IV - A (Animals Exposed to Carbogen)

Animal No.	Time	pH	PO_2(mm Hg)	PCO_2(mm Hg)	Mean Values
1	Before Gas	7.37	60	20	pH = 7.38
2	"	7.34	60	27	PO_2 = 64 mm Hg
3	"	7.40	75	22	PCO_2 = 24 mm Hg
4	"	7.40	65	26	
5	"	7.38	60	25	
2	After 30"	7.3	220	37	pH = 7.28
4	"	7.28	200	30	PO_2 = 230 mm Hg
5	"	7.28	270	40	PCO_2 = 36 mm Hg
1	After 60"	7.30	190	37	pH = 7.31
3	"	7.32	180	33	PO_2 = 185 mm Hg
					PCO_2 = 35 mm Hg
4	After 4 Hrs.	7.23	270	42	pH = 7.26
5	"	7.30	270	38	PO_2 = 270 mm Hg
					PCO_2 = 40 mm Hg
5	30" of Room Air Recovery	7.50	65	18	

TABLE IV - B (Animals Exposed to Normal Air)

Animal No.	Time	pH	PO_2(mm Hg)	PCO_2(mm Hg)	Mean Values
1	Before Gas	7.40	60	30	pH = 7.4
2	"	7.40	60	30	PO_2 = 60 mm Hg
					PCO_2 = 30 mm Hg
1	After 30"	7.40	55	30	pH = 7.4
2	"	7.40	60	30	PO_2 = 57 mm Hg
					PCO_2 = 30 mm Hg
1	After 4 Hrs.	7.40	55	28	pH = 7.43
2	"	7.45	70	23	PO_2 = 63 mm Hg
					PCO_2 = 26 mm Hg

30 minutes of exposure to carbogen and to 40 mm Hg after 4 hours of carbogen. After 30 minutes of recovery in room air the arterial PCO_2 dropped to 18 mm Hg. Arterial PO_2 showed a dramatic rise during inhalation of carbogen. Before placement arterial PO_2 averaged 64 mm Hg. This value climbed to 230 mm Hg after 30 minutes of carbogen respiration but then dropped to 185 mm Hg after 60 minutes of exposure. At the end of 4 hours of gas exposure the arterial PO_2 was approximately 270 mm Hg. These elevated arterial PO_2 values dropped quickly to normal after 30 minutes of recovery in room air. Visual observation of animals in Experiment I revealed a rapid rise in respiratory rate immediately after placement into the carbogen atmosphere.

Arterial blood gas values from animals exposed only to the compressed normal air conditions showed no shifts in pH, in PCO_2, or in PO_2 over the course of a 4-hour exposure in the wooden cage. The values were very stable at pH of 7.4; PO_2 of 55-70 mm Hg and PCO_2 of 23-30 mm Hg.

RESULTS

EXPERIMENT II

General Findings

Carbogen inhalation prior to and during a pure tone acoustic exposure was found to substantially limit the degree of N_1 response depression relative to that observed in animals which did not receive this gas. The beneficial effects of carbogen inhalation were confined to those frequencies which exhibited the largest degree of N_1 response depression following the acoustic overload. There was no evidence that carbogen inhalation prior to and during a pure tone acoustic exposure influenced the number of frequencies affected by the overload. Additionally, there was no evidence that carbogen inhalation influenced the absolute rate of recovery of the N_1 response relative to animals which received room air. As a consequence of less N_1 response depression in the animals which received carbogen but a comparable rate of recovery between the two groups, the final level of N_1 response recovery after 3 hours was greater in the group which received carbogen than in the group which did not.

Effects of Carbogen Inhalation

Complete tables of N_1 response amplitudes at each test frequency (within each test sequence for each experimental animal) are presented in APPENDIX I. Each value in each table represents

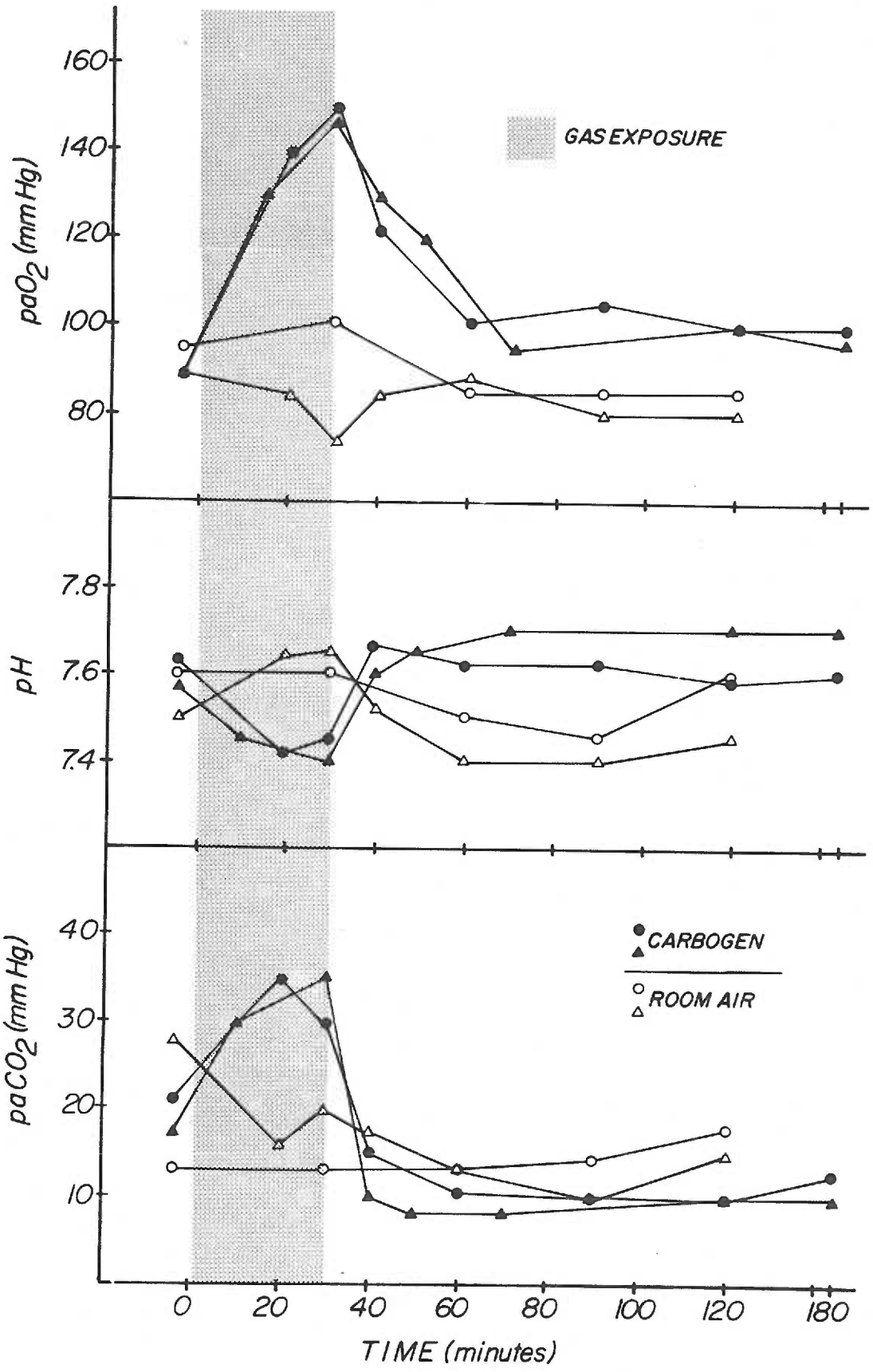
N_1 response amplitude as a percentage value relative to the mean N_1 response amplitude before acoustic overload.

It should first be noted in reference to APPENDIX I and the first 3 N_1 response measures in Figures 24-27 that the initial 20 minutes of carbogen inhalation produced no dramatic or consistent effects on N_1 response amplitude. That is, N_1 response amplitude in the animals which received either carbogen or room air before acoustic overload were similar in TS-3 to those in TS-1 and TS-2.

The effects of a 30 minute period of carbogen inhalation during continuous artificial ventilation on arterial blood pH, PCO_2 and PO_2 are shown in Figure 12. It is evident from these figures that carbogen inhalation induced a rapid rise in PO_2 and PCO_2 with a corresponding fall in pH which reached a maximum at the end of the 30 minute period. At this time the arterial pH was approximately 7.42, the PO_2 = 150 mm Hz, and the PCO_2 = 32 mm Hg. This represents approximately a 170% increase in PO_2, a 160% increase in PCO_2 and a drop in pH from 7.57 to 7.42 relative to initial measures.

It should be noted that after cessation of the carbogen inhalation arterial PCO_2 and pH decreased and increased respectively to exceed control values. This may reflect the participation of a compensatory mechanism. No such compensatory changes were observed in arterial PO_2. A further observation is that initial arterial blood gas values in both the animals that received carbogen and the animals that did not are different from the suggested normals for the guinea pig (see end of Introduction) (1). In reference to these values the pH and PCO_2 measured before gas

Figure 12. Arterial blood pH, PO_2 and PCO_2 obtained from the carotid artery of 4 guinea pigs during artificial ventilation with either Carbogen (95% O_2/5% CO_2) or normal air (21% O_2). Shaded area represents the period during which gas exposure was given. Normal room air was given at all other times.


GAS EXPOSURE
paO_2 (mm Hg)
160
140
120
100
80
pH
7.8
7.6
7.4
$paCO_2$ (mm Hg)
40
30
20
10
CARBOGEN
ROOM AIR
0
20
40
60
80
100
120
180
TIME (minutes)

exposure represent alkalosis and hypercapnia Further, the pH in the animals given only room air decreased over the 120 minute period as did the PCO_2 in one animal. The possible mechanisms behind this observed alkalosis and hypercapnia and its implications will be discussed.

Electrophysiological Findings

In all animals the range of frequencies showing the most pronounced N_1 response depression following the acoustic overload were found above the overload frequency (4.5 kHz). The frequency or frequencies showing the maximum amount of N_1 response suppression were found approximately 1/2 to 3/4 of an octave above this (6.4 - 7.6 kHz) in all animals regardless of gas exposure condition. This finding is consistent with other reports of pure tone induced N_1 response depression (69)(76).

There was a large degree of variability in the degree of depression and the width of the range of frequencies affected from animal to animal within the same group and between animals in the two groups (APPENDIX I). In some ears, N_1 responses 20 seconds after the acoustic overload were suppressed to 0.0 % from 4.9 kHz (frequency 11) through 9.9 kHz (frequency 19). In other ears this range of maximally depressed frequencies was much more narrow. In some animals N_1 responses never reached 0.0% after the acoustic overload. In general, a consistent relationship between the degree of depression and the range of depressed frequencies was observed between each pair of matched animals (TABLE II). In each

case carbogen was found to have led to a reduction in the degree of N_1 response depression without much difference in the range of frequencies showing effects of the acoustic trauma.

A sample Test Sequence array of N_1 responses at 32 test frequencies (APPENDIX B) taken 21 seconds after cessation of the acoustic overload is shown in Figure 13. This TS-4 is from the same animal previously presented in Figure 6 before acoustic overload. In TS-4 the area of maximum N_1 response depression and the range of frequencies affected are clearly visible.

A sample TS (TS-5) taken after 2 minutes of recovery in the same animal shows a partial recovery of N_1 response amplitude at all test frequencies (Figure 14). After 5 minutes of recovery (Figure 15) all frequencies show an N_1 response in this animal. The last sequence of figures serve only to illustrate the frequency specific effects of pure-tone induced depression of the N_1 response and the basis for the following data.

Relative Depression of Frequencies-Raw Data Animals G.P.#1 and G.P.#4

Figure 16 shows the N_1 response amplitude from two matched animals at each of the 32 test frequencies expressed as percentage values relative to the mean N_1 response amplitude in the 3 Test Sequences before acoustic overload. Four TS's are shown which represent measures at 20 seconds, 2 minutes, 5 minutes and 10 minutes after cessation of the acoustic overload.

It may be seen in Figure 16 that the animal which received

Figure 13. Sample array of averaged N_1 responses at 32 test frequencies from 2.1 kHz through 30 kHz taken 21 seconds after cessation of the acoustic overload (TS-4). Note that the range of frequencies showing N_1 response supression extends from approximately frequency #12 through frequency #16 (5.4-9.1 kHz). Frequency integers are in 1/8 octave intervals from 2.1 kHz through 30 kHz (APPENDIX B). Overload frequency was #10 (4.5 kHz).

SAMPLE TS N1 RECOVERY G. P. (BIOCHEMOGEN), TIME POST-EXP= TS 4 21 SEC

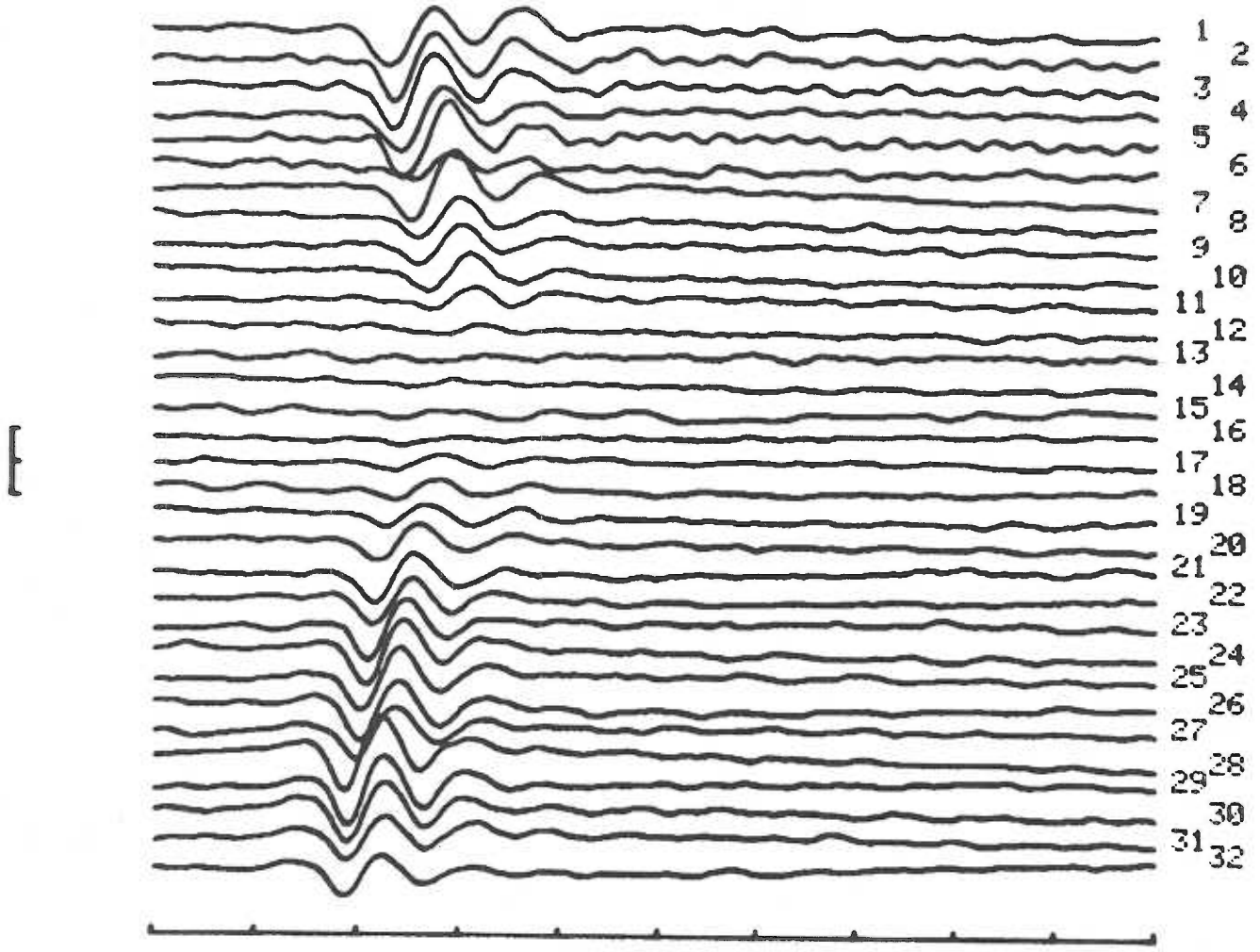


Figure 14. Sample array of averaged N_1 responses at 32 frequencies from 2.1 kHz through 30 kHz taken 121 seconds after cessation of the acoustic overload (TS-5). Note recovery of N_1 responses at frequencies #12 through #18 relative to the preceeding figure. Frequency intervals are in 1/8 octaves from 2.1 kHz through 30 kHz. Overload was frequency #10 (4.5 kHz).

SAMPLE TS N1 RECOVERY G.P.#10(CARBOGEN) TIME POST-EXP= TS 5 121 SEC

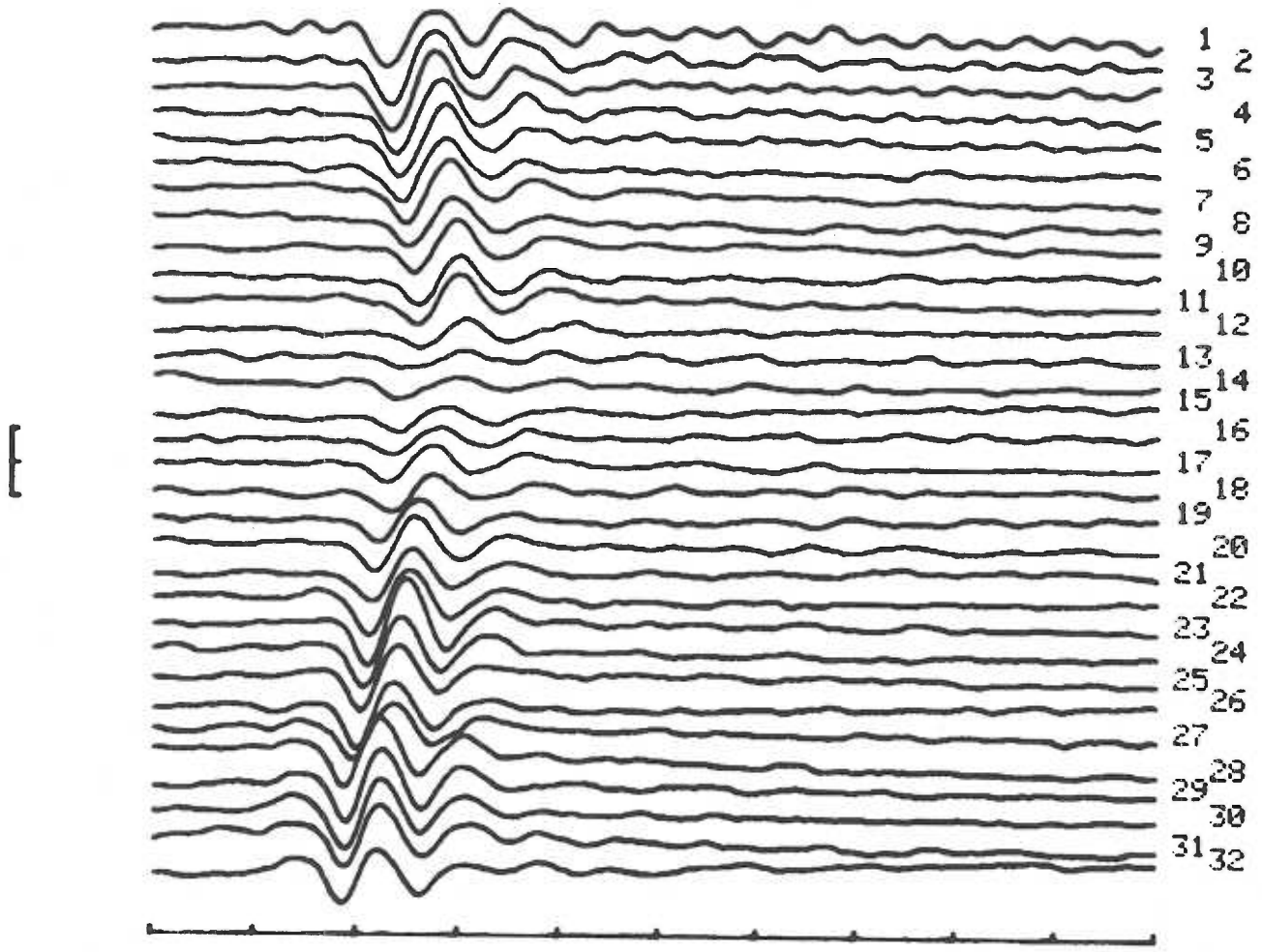


Figure 15. Sample array of averaged N_1 responses at 32 frequencies from 2.1 kHz through 30 kHz taken 905 seconds after cessation of the acoustic overload (TS-8). Note robust N_1 response at all test frequencies relative to preceeding figures. Frequency intervals are in 1/8 octaves from 2.1 kHz through 30 kHz. Overload was frequency #10 (4.5 kHz).

SAMPLE TS N1 RECOVERY G.P.#10 CARBOGEN ,, TIME POST-EXP= TS 8 905 SEC

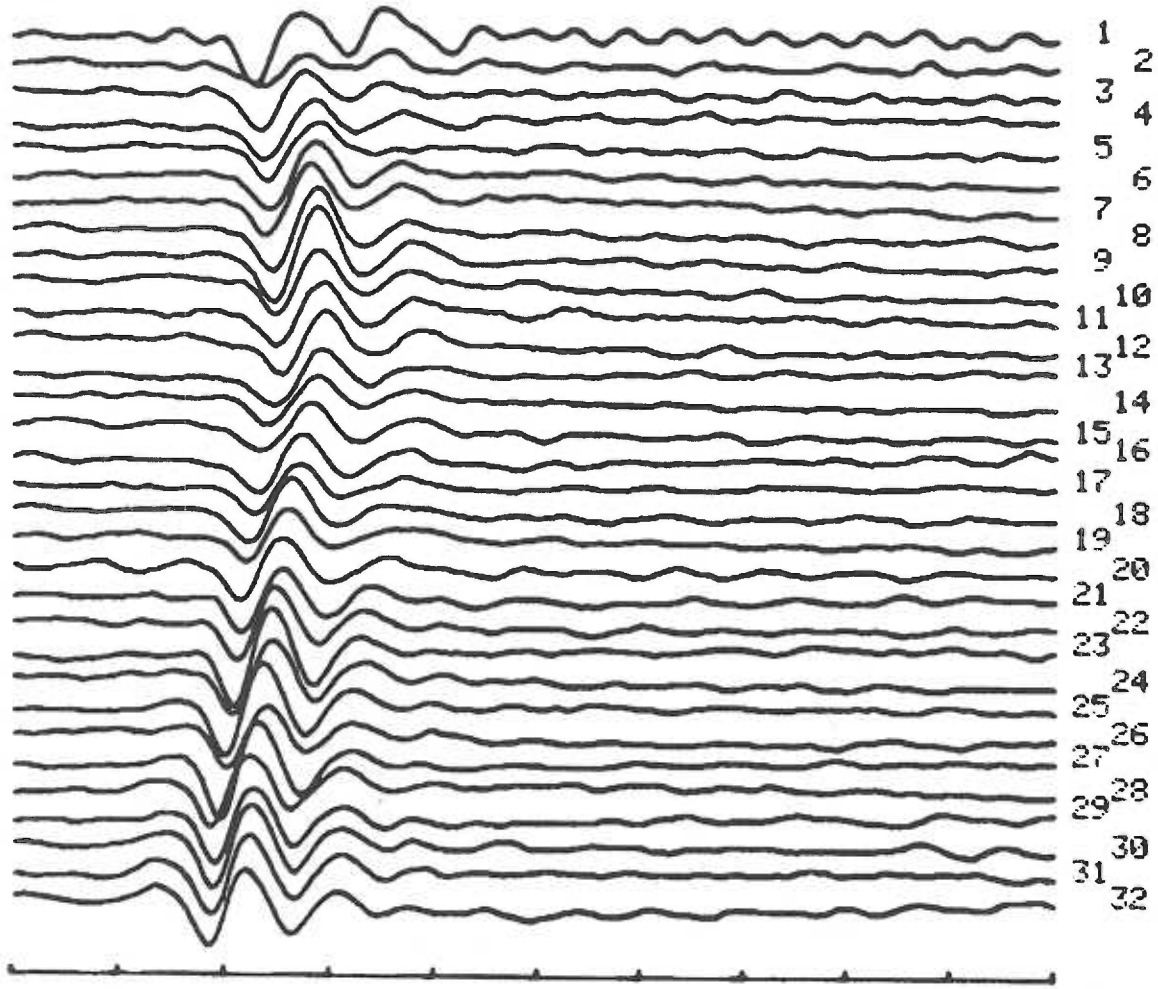
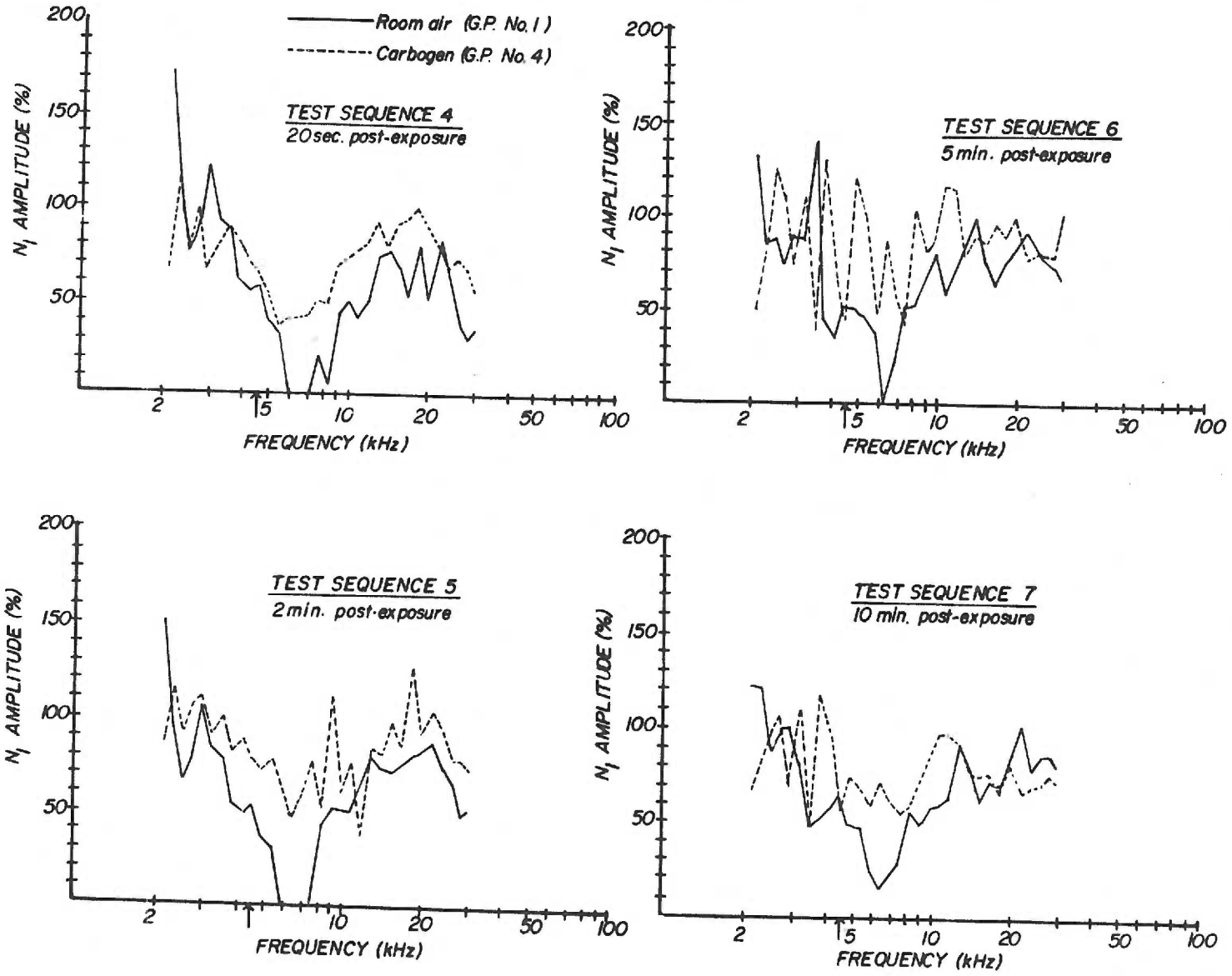


Figure 16. Sample relative depression (RD) curves showing N_1 response amplitude expressed as a percentage value relative to mean pre-exposure N_1 response amplitude for each ear. N_1 responses were measured at various times following a 4.5 kHz, 104 dB pure tone acoustic overload (arrow on horizontal axis). The two curves in each diagram represent raw data from a pair of matched animals one of which received carbogen during acoustic overload the other of which received room air.


Room air (G.P. No. 1)
Carbogen (G.P. No. 4)
TEST SEQUENCE 4
20 sec. post-exposure
TEST SEQUENCE 6
5 min. post-exposure
TEST SEQUENCE 5
2 min. post-exposure
TEST SEQUENCE 7
10 min. post-exposure
N1 AMPLITUDE (%)
FREQUENCY (kHz)
200
150
100
50
2
5
10
20
50
100

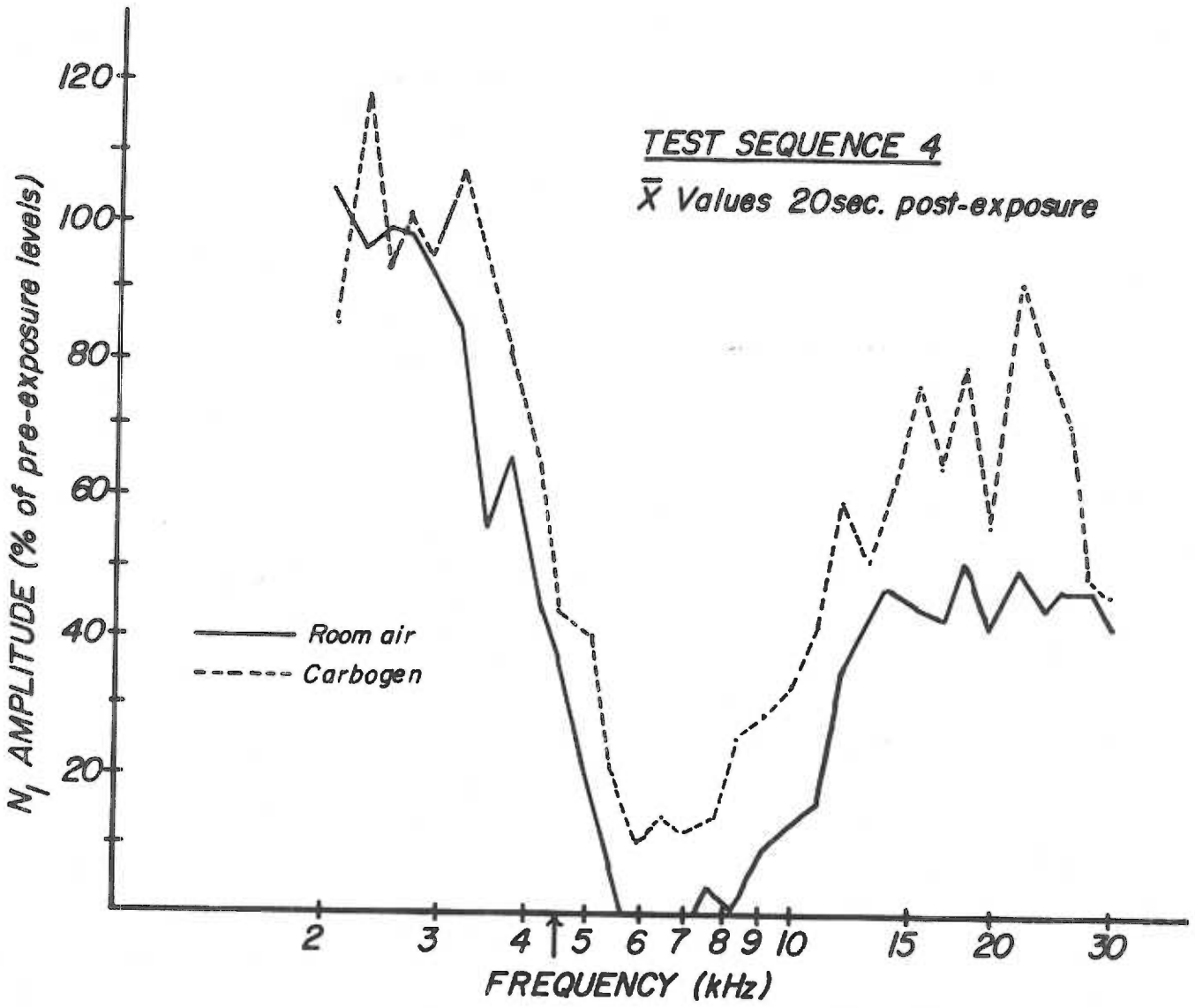
carbogen (G.P. #4) gas for 20 minutes prior to and then 10 minutes during the acoustic overload demonstrated less N_1 response depression than his appropriate control (G.P. #1) in each relative depression (RD) plot. The effects of carbogen inhalation were only evident where there was substantial N_1 response depression. That is, where no depression occurred, no difference between the animals is present in N_1 response amplitude. The areas of maximum N_1 response depression were found approximately 1/2 to 1 octave above the overload frequency (indicated by the small arrows on the horizontal axis). Over the recovery period encompassed by these RD curves both ears demonstrated substantial N_1 response recovery. The beneficial effects of carbogen inhalation are still quite evident in TS-7.

A further observation concerning the raw data presented in the preceeding figure is that while depression of N_1 response amplitude was more pronounced in the animal which received room air than in the animal which received carbogen during acoustic overload, the range of frequencies which were affected by the pure-tone overload does not appear to differ dramatically. Apart from a difference in degree of N_1 response depression, the general shape of the RD curves in each TS is about the same. This observation becomes more evident when mean RD curves which include all animals within each treatment group are seen.

Relative Depression of Frequencies-Mean Data

Figure 17 shows the mean RD curves from two groups of 5 animals

Figure 17. Mean relative depression (RD) curves obtained from all ears in two groups of animals in which N_1 response amplitudes at each frequency are expressed as percentage values relative to mean pre-exposure N_1 response amplitudes at that frequency. Test Sequence-4 was taken 20 seconds after cessation of the acoustic overload. Note that the maximum depression of the N_1 response occured approximately one-half octave above the exposure frequency (indicated by the arrow on the horizontal axis) in both the group which received room air and the group which received carbogen during acoustic overload.

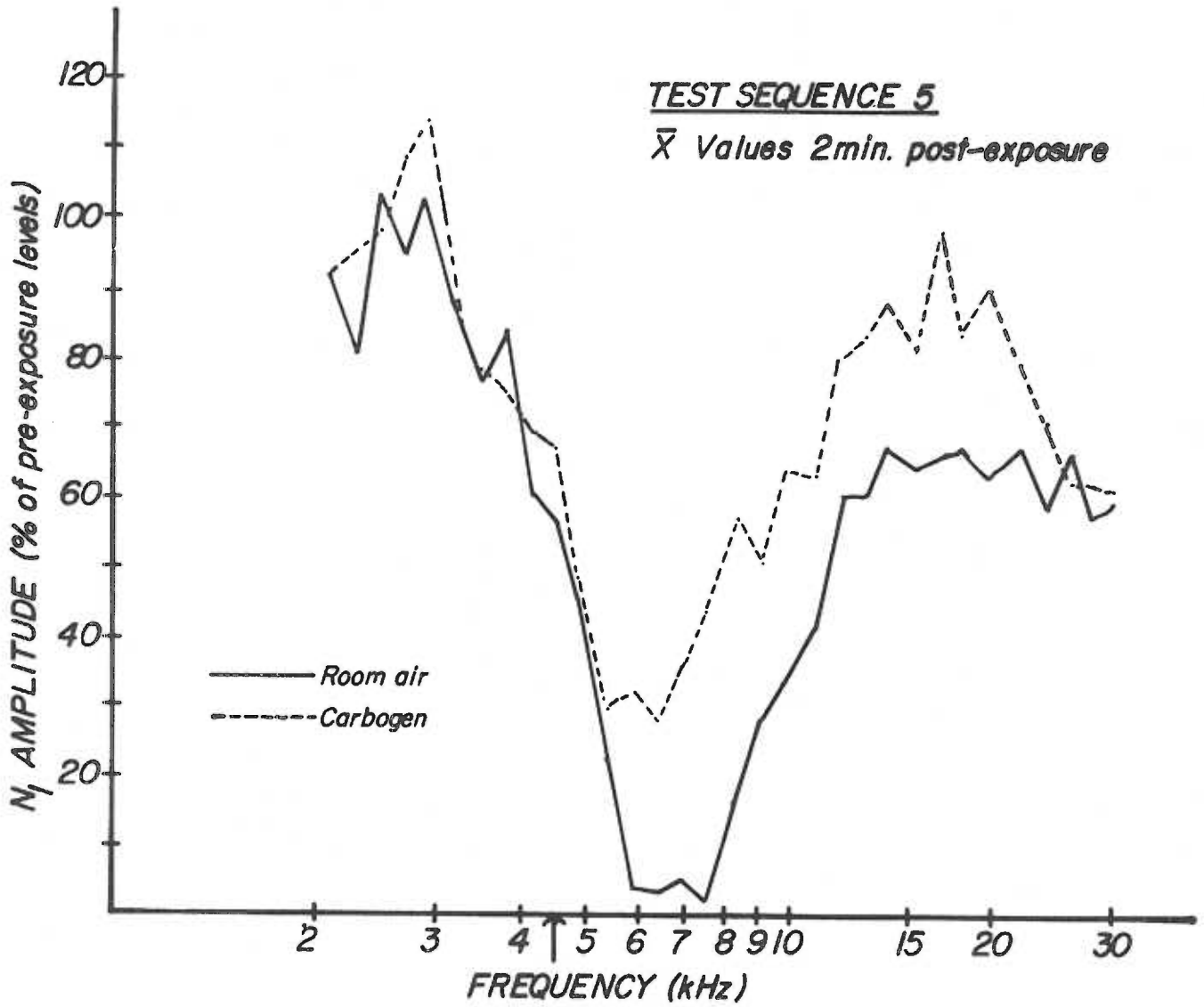

TEST SEQUENCE 4
$\bar{X}$ Values 20sec. post-exposure
N_1 AMPLITUDE (% of pre-exposure levels)
120
100
80
60
40
20
Room air
Carbogen
2
3
4
5
6
7
8
9
10
15
20
30
FREQUENCY (kHz)

in Test Sequence-4; taken 20 seconds after cessation of the acoustic overload. The relative lack of N_1 response amplitude depression in the group of animals which received carbogen during exposure is clearly evident in this figure. The similarity in the shape of the RD curves from the two groups of animals is also evident as is the similarity in the frequency which demonstrated the maximum N_1 response depression. It should be noted that there was a large beneficial effect on the degree of N_1 response depression as a result of carbogen inhalation Within the maximally depressed frequency range, the group which received room air during acoustic exposure demonstrated N_1 response amplitudes that reach a value of 0.0 % while the group which received carbogen did not.

Further observation of these mean RD curves reveals that the range of frequencies above 15 kHz also show some differences in N_1 response amplitude between the two experimental groups. It should be noted that the Room Air Group showed substantial depression relative to the Carbogen Group despite the fact that this frequency range lies far above the area of maximum N_1 response depression. The relative exacerbation of depression in this high frequency range in the Room Air Group persists up to 2 minutes following cessation of the acoustic overload and then does not subsequently appear.

Figure 18 shows mean RD curves from the two experimental groups of animals 2 minutes after cessation of the acoustic overload. Because the N_1 response depression in the group which received room air does not reach a measurable minimum of 0.0 % the effects

Figure 18. Mean relative depression (RD) curves obtained from all ears in two groups of animals in which N_1 response amplitude at each test frequency is expressed as a percentage value relative to the mean pre-exposure N_1 response amplitude at that frequency. Test Sequence-5 was taken 2 minutes after cessation of the acoustic overload.

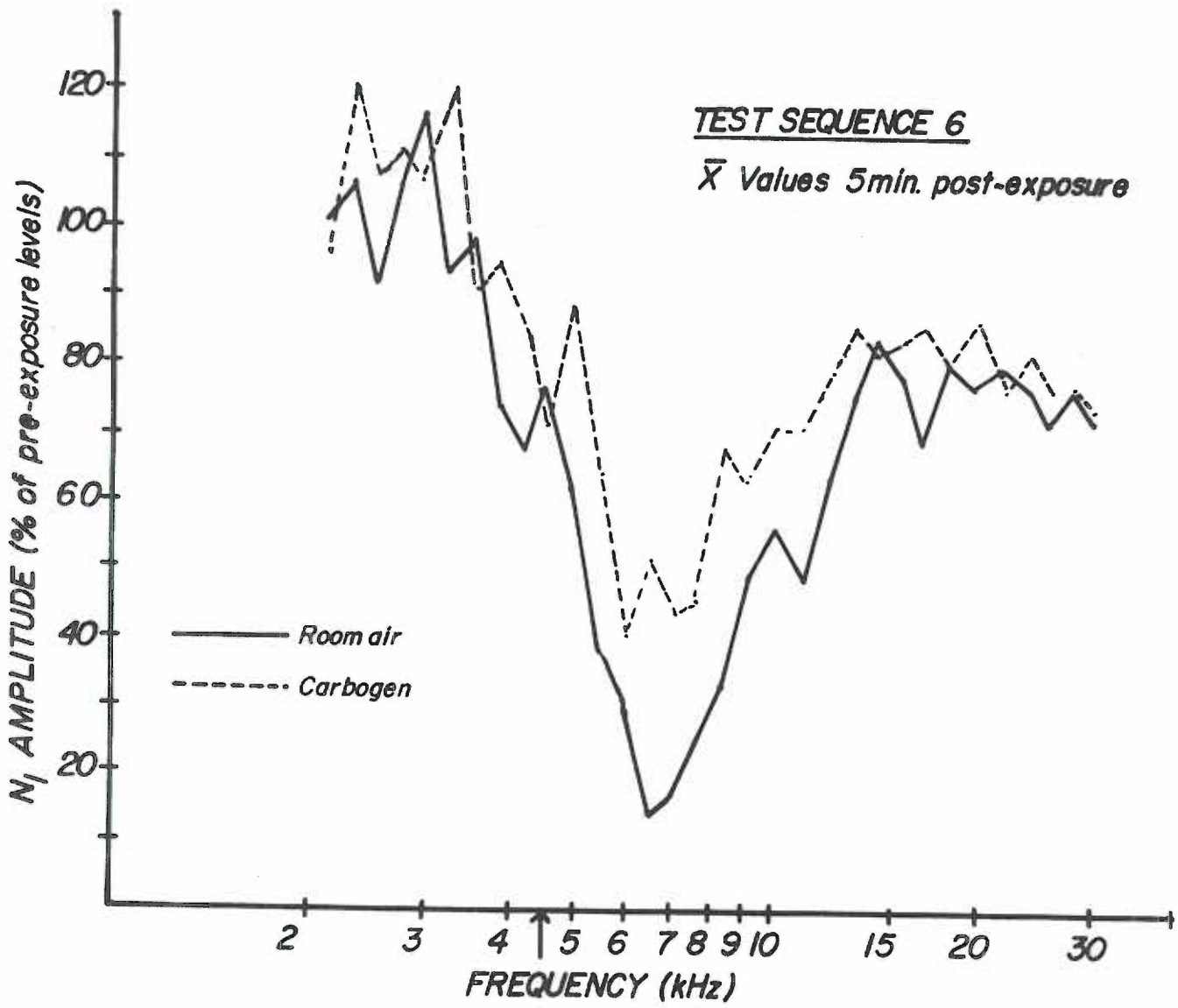

TEST SEQUENCE 5
$\bar{X}$ Values 2min. post-exposure
N_1 AMPLITUDE (% of pre-exposure levels)
120
100
80
60
40
20
Room air
Carbogen
2
3
4
5
6
7
8
9
10
15
20
30
FREQUENCY (kHz)

of carbogen on induced N_1 depression are more clearly evident than in Figure 17. Examination of Figure 18 reveals that approximately 25% difference exists between the two experimental groups at the area of maximum N_1 suppression (approximately 6.4 kHz). Because this value is relative to the mean N_1 amplitude before acoustic overload (approximately 50 μV) this indicated that a mean difference of approximately 13 μV of N_1 response was produced by carbogen inhalation. When compared to the last figure. the Carbogen Group RD curve seems shifted somewhat to the left relative to the RD curve from the group which received room air. Again, however, the high frequencies show more damage in the latter group.

Figure 19 shows mean RD curves from the two experimental groups 5 minutes after cessation of the acoustic overload. Both groups have shown substantial recovery of N_1 response amplitude in all frequencies at this point in time. The relative difference between the two groups is still approximately 25% at the maximally depressed frequency. A major change in this TS relative to the previous two figures is that the frequencies above 15 kHz in the Room Air Group have recovered to a point equivalent in amplitude to the Carbogen Group. When the RD curves in Figure 18 and Figure 19 are compared it is evident that N_1 response amplitudes in frequencies above 15 kHz have not recovered very much in the Carbogen Group while substantial recovery has occurred in the Room Air Group.

Figure 20 shows mean RD curves 10 minutes after acoustic

Figure 19. Mean relative depression (RD) curves obtained from all ears in two groups of animals in which N_1 response amplitude is expressed as a percentage value relative to the mean pre-exposure N_1 response amplitude at each frequency. Test Sequence-6 was taken 5 minutes after cessation of the acoustic overload.


TEST SEQUENCE 6
$\bar{X}$ Values 5min. post-exposure
N_1 AMPLITUDE (% of pre-exposure levels)
120
100
80
60
40
20
Room air
Carbogen
2
3
4
5
6
7
8
9
10
15
20
30
FREQUENCY (kHz)

exposure. In this TS the two groups of animals show even less difference in the degree of N_1 response depression. At the frequency of maximum depression the two groups now differ by only 18% of pre-exposure levels or approximately 9 μV. There are no observable differences in N_1 response amplitude between the two groups in the very high frequencies and the approximate shape of the curves are the same.

Relative Recovery of Frequencies: Raw Data G.P.#1 and G.P.#4

Depression and recovery of individual frequencies following acoustic overload from a representative pair of animals are shown in Figure 21, Figure 22 and Figure 23. In each case G.P. #1 and G.P. #4 are presented. Again, values are plotted relative to mean pre-exposure N_1 amplitudes and so these curves represent relative recovery (RR) measures.

Figure 21 shows the relative recovery (RR) curves for frequency 6 (2.3 kHz); frequency 9 (4.2 kHz); frequency 10 (overload frequency, 4.5 kHz); and frequency 11 (4.9 kHz). Frequency 6 lies well below the range of maximally depressed frequencies, exactly 1/2 an octave below frequency 10. At this frequency very little N_1 response depression due to acoustic overload is evident, as would be predicted from the mean RD curves for the two groups. There is some decline in N_1 response amplitude over time in both animals regardless of gas inhalation condition.

At frequency 9, N_1 response depression is more visible. In this diagram the carbogen animal demonstrates very little N_1

Figure 20. Mean relative depression (RD) curves obtained from all ears in two groups of animals in which N_1 response amplitude is expressed as a percentage value relative to the mean pre-exposure N_1 response amplitude at each frequency. Test Sequence-7 was taken 10 minutes after cessation of the acoustic overload.

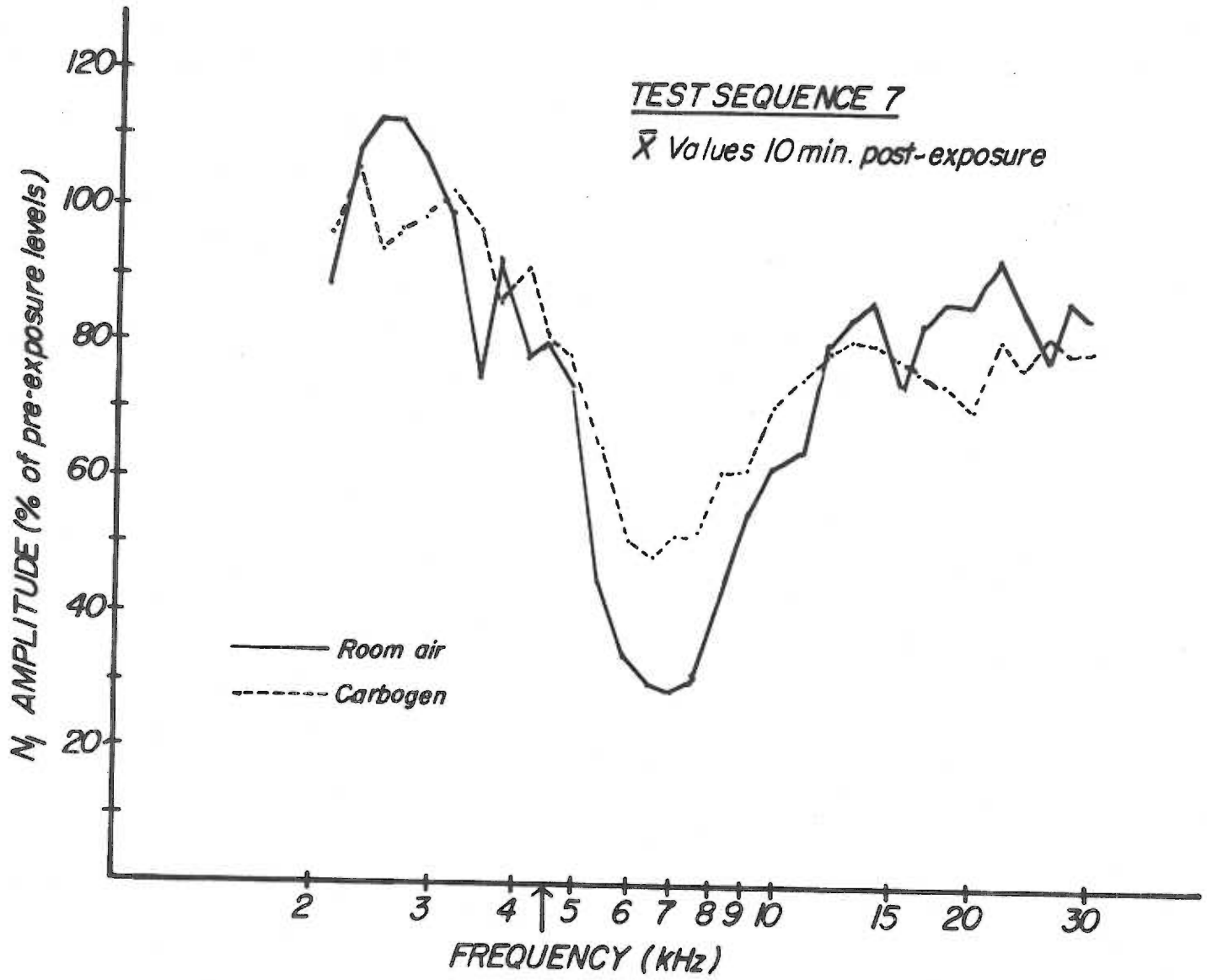
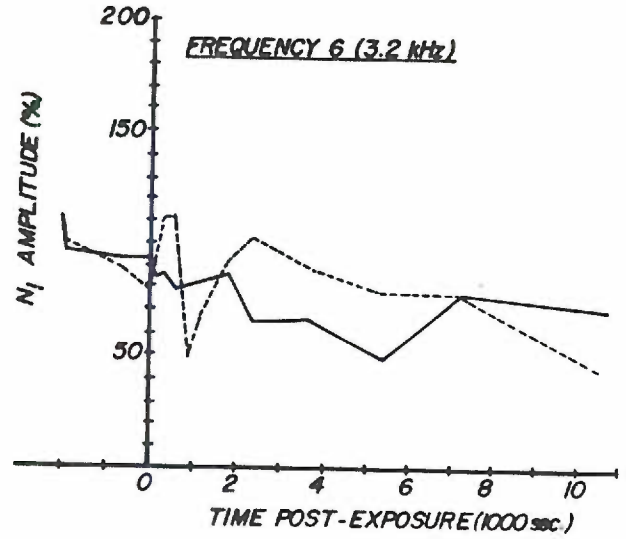
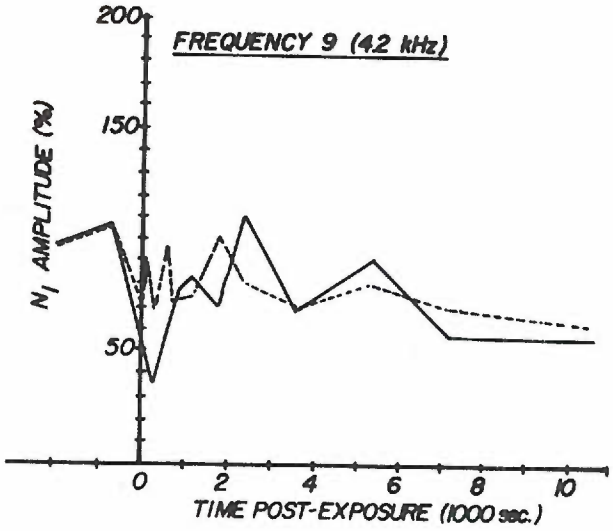
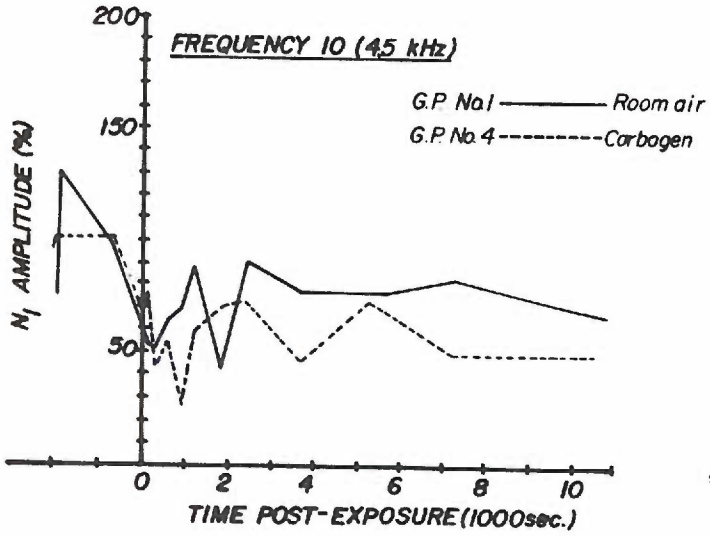
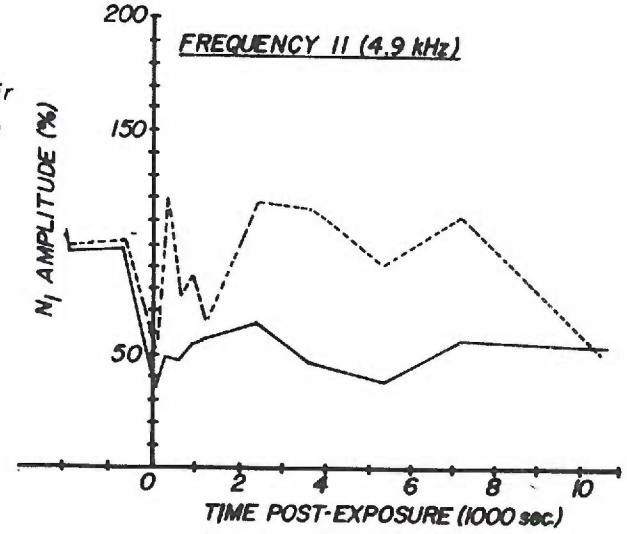

TEST SEQUENCE 7
$\bar{X}$ Values 10 min. post-exposure
N_1 AMPLITUDE (% of pre-exposure levels)
120
100
80
60
40
20
Room air
Carbogen
2
3
4
5
6
7
8
9
10
15
20
30
FREQUENCY (kHz)

Figure 21. Sample relative recovery (RR) curves showing N_1 response amplitude expressed as a percentage value relative to mean pre-exposure levels. The vertical axis represents the time of cessation of the acoustic overload. Depression and recovery of four frequencies are shown. Data are taken from a matched pair of animals which received either carbogen (G.P. #4) or room air (G.P. #1) during acoustic overload.


FREQUENCY 6 (3.2 kHz)
200
150
50
N_1 AMPLITUDE (%)
0
2
4
6
8
10
TIME POST-EXPOSURE (1000 sec.)


FREQUENCY 9 (4.2 kHz)
200
150
50
N_1 AMPLITUDE (%)
0
2
4
6
8
10
TIME POST-EXPOSURE (1000 sec.)


FREQUENCY 10 (4.5 kHz)
G.P. No.1 ——— Room air
G.P. No.4 - - - - - - - - Carbogen
200
150
50
N_1 AMPLITUDE (%)
0
2
4
6
8
10
TIME POST-EXPOSURE (1000 sec.)


FREQUENCY 11 (4.9 kHz)
200
150
50
N_1 AMPLITUDE (%)
0
2
4
6
8
10
TIME POST-EXPOSURE (1000 sec.)

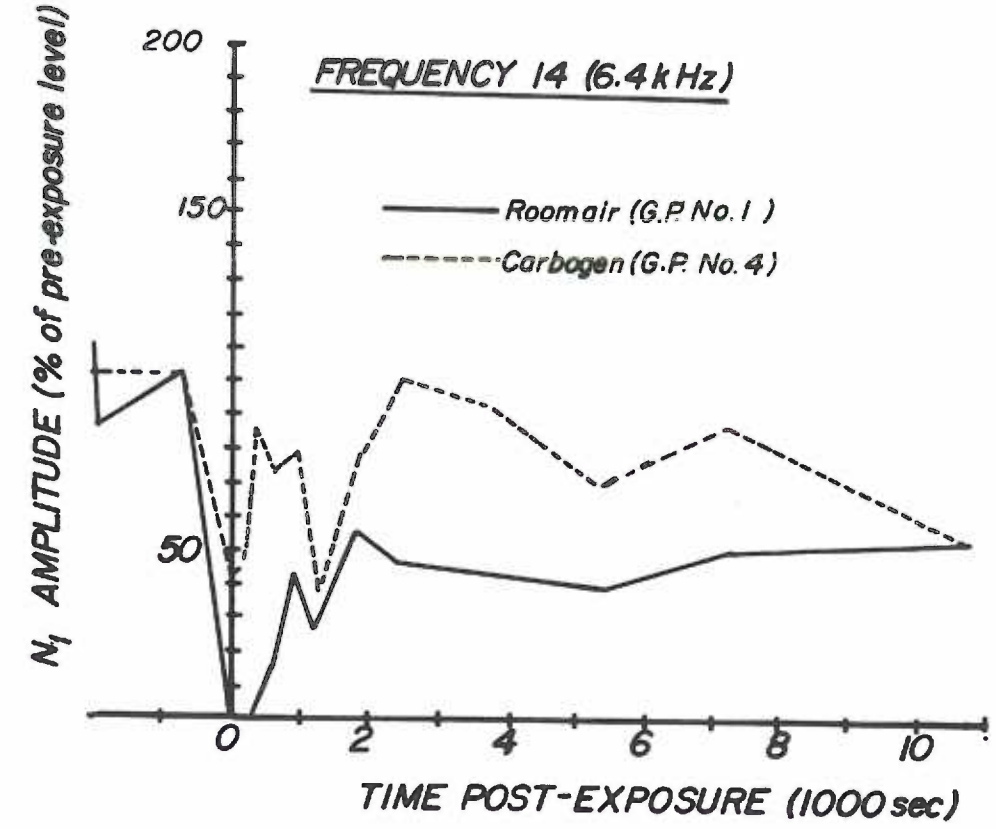
response depression while the room air animal showed a nearly 30% (15 μV) decrease in amplitude. Recovery of responses at this frequency was very rapid and after a few minutes both animals exhibit similar N_1 response amplitudes.

Frequency 10 exhibits effects similar to those observed at frequency 9 whereas frequency 11 does not. This latter frequency approaches the maximally depressed range. At frequency 11 very distinct differences in the degree of N_1 response depression were found between the two gas conditions. As has been pointed out, such differences in relative N_1 depression occurred primarily above the exposure frequency.

Figure 22 shows RR curves from G.P. #1 and G.P. #4 at frequency 14 and frequency 15 in the center of the range of frequencies which showed maximum N_1 response depression (6.4 and 6.9 kHz respectively). In both diagrams the effects of the carbogen are very evident. The degree of N_1 response depression is far less in the animal which received carbogen, especially in the first 1000 seconds after acoustic overload. After this time, differences between the two groups are less pronounced.

Figure 23 presents the RR curves from the representative ears for frequencies 17 (8.3 kHz); frequency 18 (9.1 kHz); frequency 19 (9.9 kHz); and frequency 24 (15.3 kHz). Frequency 17 lies just below the full octave above the exposure frequency. The effects of carbogen are evident at this frequency, again in terms of degree of depression and initial stages of recovery. Frequency 18 shows similar effects. At frequency 19, toward the

Figure 22. Sample relative recovery (RR) curves showing N_1 response amplitude as a percentage value relative to mean pre-exposure levels. The vertical axis represents the time of cessation of the acoustic overload. Depression and recovery of two frequencies are shown which are one-half and five-eights of an octave above the exposure frequency. Data are taken from a matched pair of animals.


FREQUENCY 14 (6.4 kHz)
Room air (G.P. No. 1)
Carbogen (G.P. No. 4)
N_1 AMPLITUDE (% of pre-exposure level)
200
150
50
0
2
4
6
8
10
TIME POST-EXPOSURE (1000 sec)

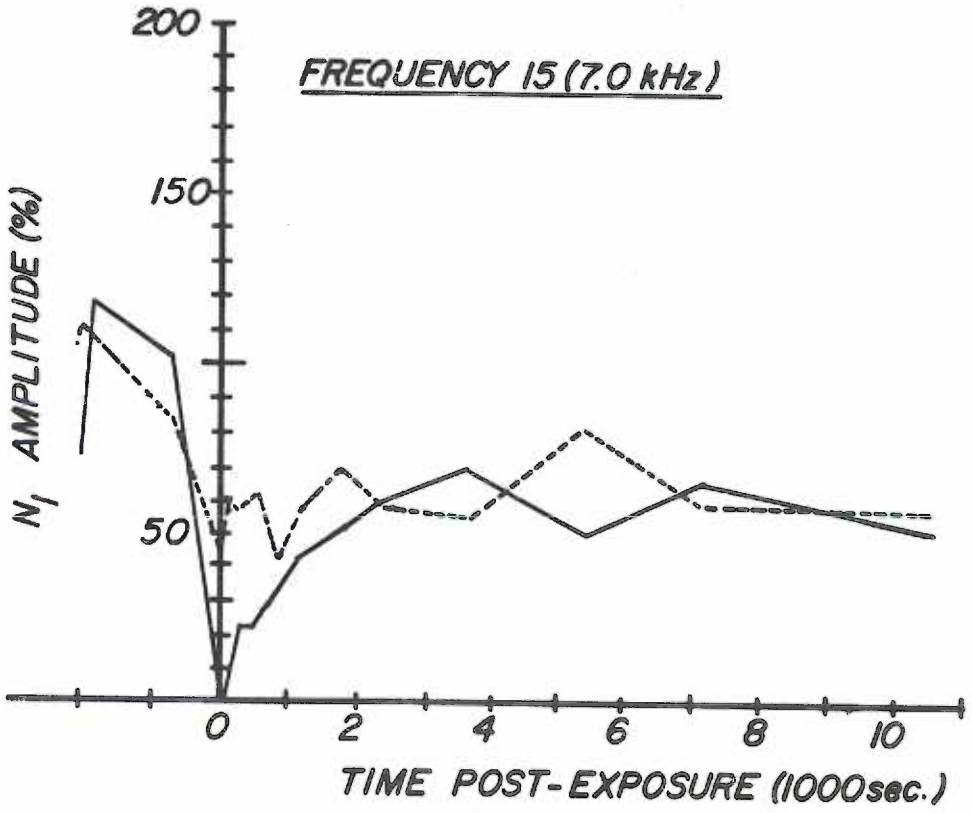
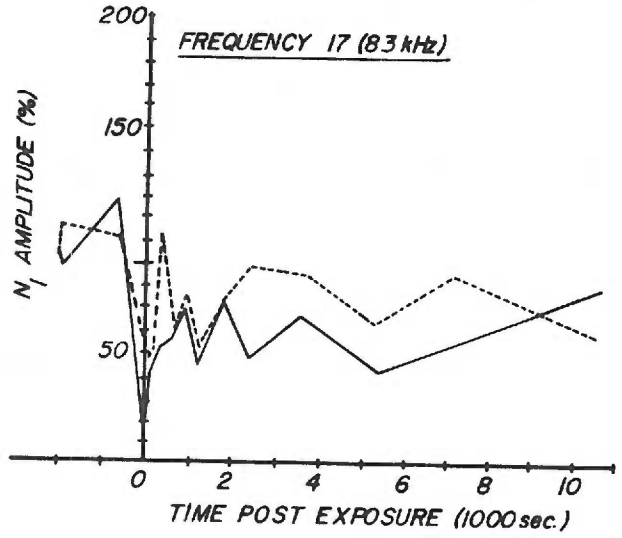
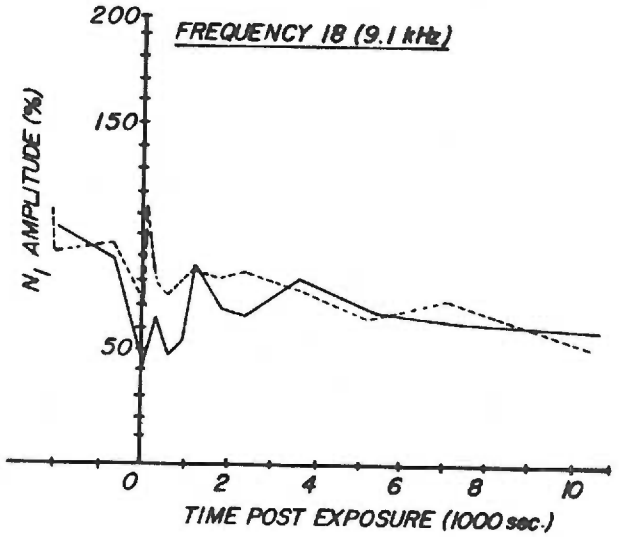
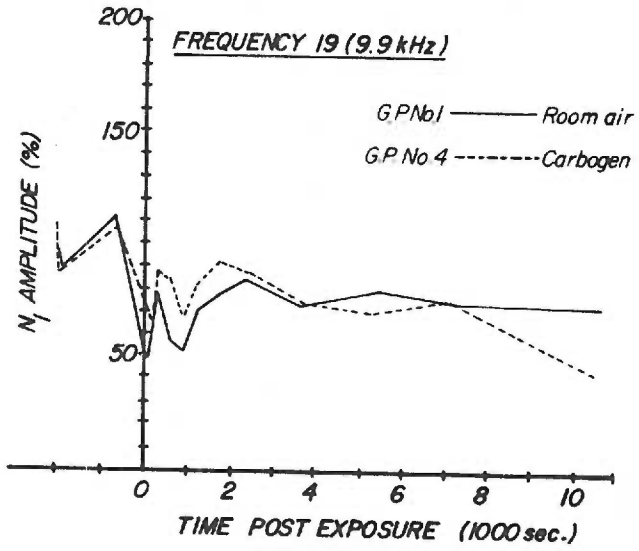
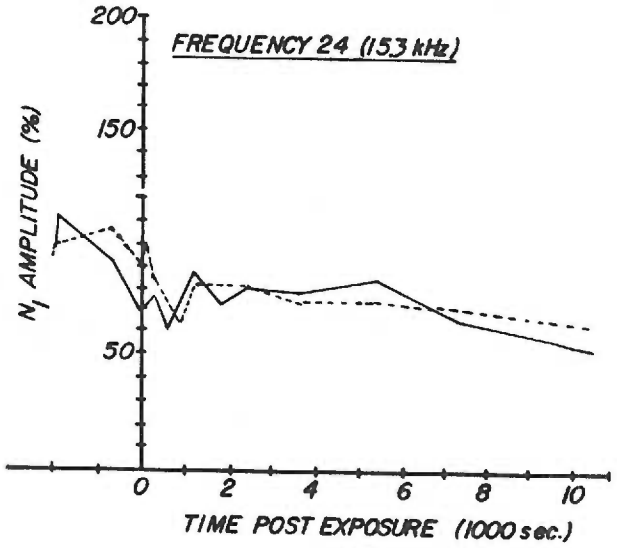

FREQUENCY 15 (7.0 kHz)
N_1 AMPLITUDE (%)
200
150
50
0
2
4
6
8
10
TIME POST-EXPOSURE (1000 sec.)

Figure 23. Sample relative recovery (RR) curves showing N_1 response amplitude expressed as a percentage value relative to mean pre-exposure levels. Depression and recovery of four frequencies are shown. Data are taken from a matched pair of animals.


FREQUENCY 17 (8.3 kHz)
200
150
50
N_1 AMPLITUDE (%)
0
2
4
6
8
10
TIME POST EXPOSURE (1000 sec.)


FREQUENCY 18 (9.1 kHz)
200
150
50
N_1 AMPLITUDE (%)
0
2
4
6
8
10
TIME POST EXPOSURE (1000 sec.)


FREQUENCY 19 (9.9 kHz)
G.P No 1 ——— Room air
G.P No 4 - - - - - - - Carbogen
200
150
50
N_1 AMPLITUDE (%)
0
2
4
6
8
10
TIME POST EXPOSURE (1000 sec.)


FREQUENCY 24 (15.3 kHz)
200
150
50
N_1 AMPLITUDE (%)
0
2
4
6
8
10
TIME POST EXPOSURE (1000 sec.)

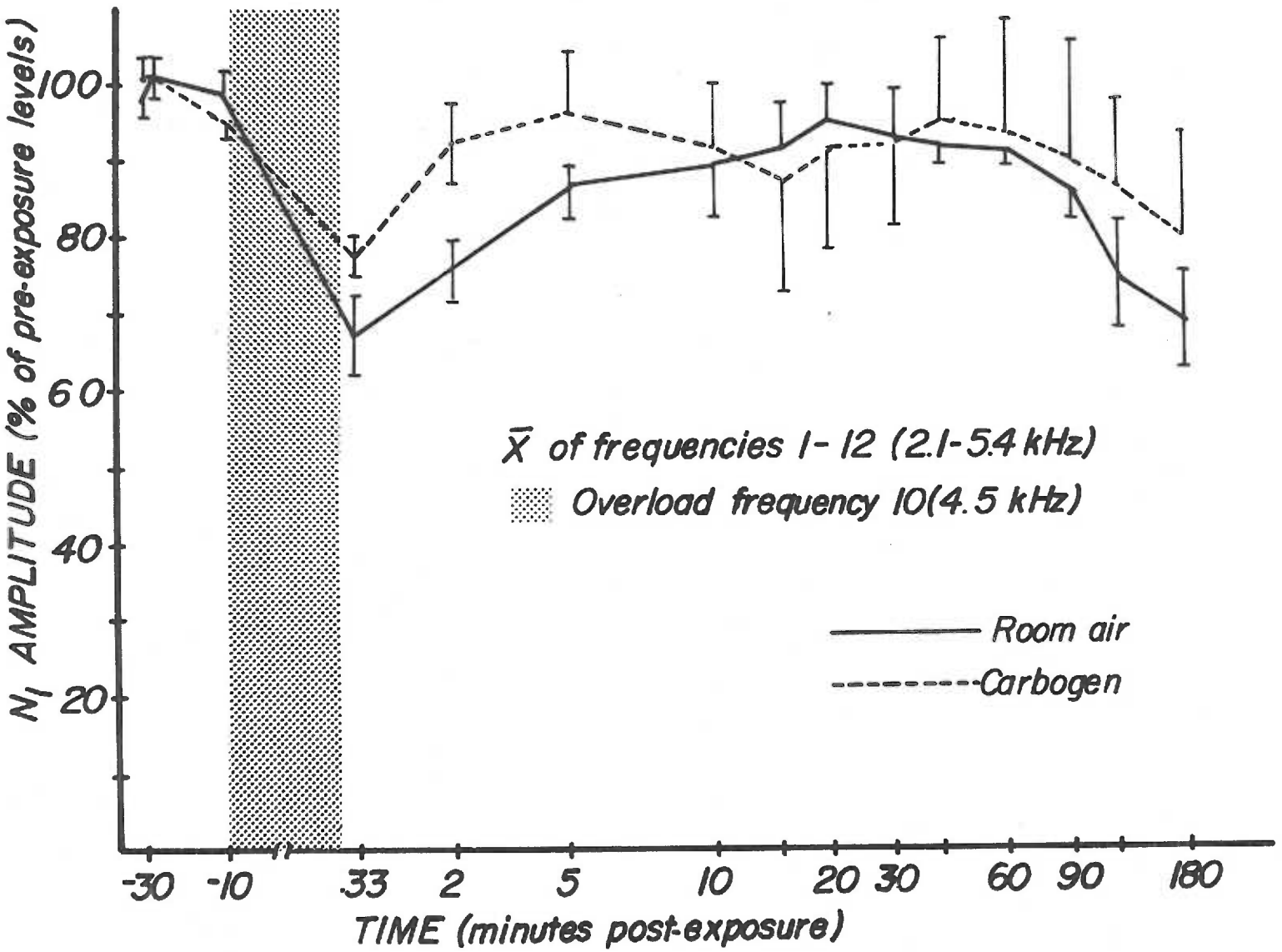
high frequency side of the maximally depressed range, very little difference in the degree of N_1 depression between the two ears is evident. This decrease in the effects of carbogen are still more pronounced at frequency 24.

As may be seen in all presentations of raw data from the representative pair of ears, the level of variability of the N_1 responses was high relative to the total N_1 amplitude measured. Because of this limitation, comparisons between each set of matched ears at each frequency provide only qualitative information. In order to provide more meaningful information and statistical analysis, pooling of data in frequency subsets provided mean RR curves for each group of animals.

Relative Recovery, Mean Data

Mean relative recovery curves for the two experimental groups are shown in Figure 24, Figure 25, Figure 26 and Figure 27. In each diagram the N_1 response amplitude is expressed as a percentage value relative to the mean N_1 response amplitude before overload. The frequency set 1-12 (2.1 kHz - 5.4 kHz) is shown in Figure 24. This range of frequencies lies generally below the area of maximum N_1 depression with the exception of frequency 11 and frequency 12. It is evident from this figure that some depression of N_1 response occurred as a result of the acoustic exposure. This depression is present in the first minutes after acoustic overload and showed quick recovery. It should be pointed out that N_1 recovery in this frequency range was faster in the

Figure 24. Mean relative recovery (RR) curves obtained from all ears in two groups of animals showing recovery of the N_1 response following an acoustic overload in which N_1 response amplitude is expressed as a percentage value relative to mean pre-exposure levels for each subset of frequencies. Vertical bars represent ± 1 SEM. The stippled area represents the duration of the acoustic overload (10 minutes). Frequencies #1-#12 (2.1-5.4 kHz) are shown.


N1 AMPLITUDE (% of pre-exposure levels)
100
80
60
40
20
X̄ of frequencies 1-12 (2.1-5.4 kHz)
Overload frequency 10(4.5 kHz)
Room air
Carbogen
-30
-10
.33
2
5
10
20
30
60
90
180
TIME (minutes post-exposure)

group which received room air than in the group which received carbogen. Both groups show equivalent N_1 response depression after 10 minutes of recovery. The depression observed in this frequency range is most probably due to that in frequencies 11 and 12. The analysis of variance applied to these data fails to show any significant differences in the degree of N_1 depression and recovery $F_{(1,8)} = 0.26$ (APPENDIX J) over the 3 hour recovery period.

Figure 25 shows the mean RR curves for the frequencies which lie above the range of maximally depressed N_1 responses (frequencies 19 through 32 (9.9 kHz - 30 kHz). As with the lower frequency subset some depression as a result of the acoustic overload was present. Again, no differences between the curves over the 3 hour period were found by statistical analysis $F_{(1,8)} = 0.22$. It should be noted that the degree of N_1 depression does appear to differ between the two groups in the first five minutes after overload as was seen in the mean RD curves within this time period. After 5 minutes the curves intersect. It is also interesting to note that the maximum recovery of N_1 responses in this frequency range did not occur until 40 minutes after the acoustic overload whereas maximum recovery in the previous figure occurred between 5 and 10 minutes after overload.

Figure 26 shows the mean RR curves for the frequencies 13-18 (5.9 kHz - 9.1 kHz). This frequency range encompasses the maximally depressed frequencies evident in the mean RD curves already shown. In contrast to the mean RR curves at the frequencies above and below this frequency range, frequencies 13-18 do differ significantly ($F_{(1,8)} = 8.39$, $p<.05$). The group which received

Figure 25. Mean relative recovery (RR) curves obtained from all ears in two groups of animals showing recovery of the N_1 response following an acoustic overload in which N_1 response amplitude is expressed as a percentage value relative to mean pre-exposure levels for each subset of frequencies. Vertical bars represent ± 1 SEM. Frequencies #19-#32 (9.9-30 kHz) are shown.

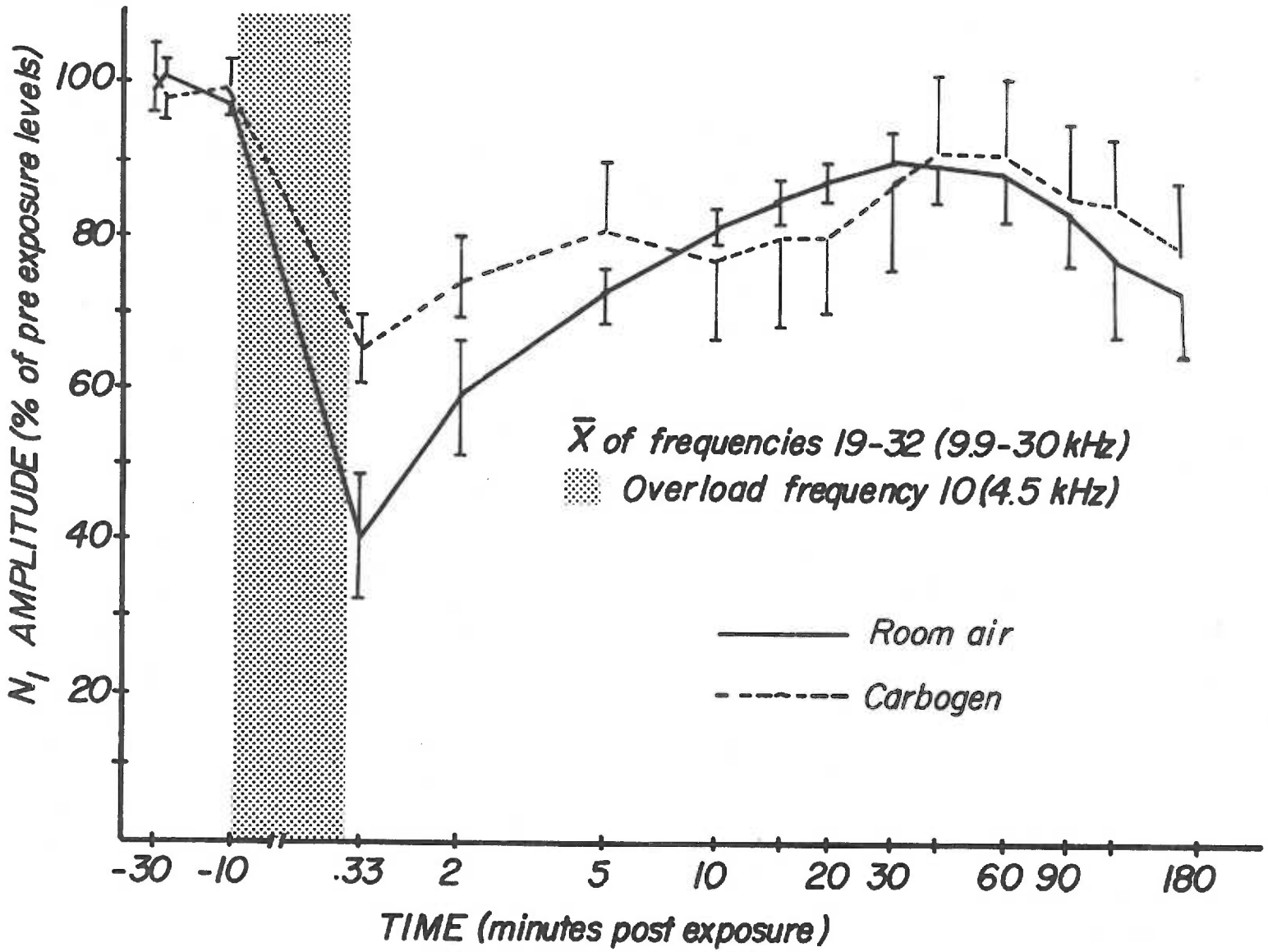
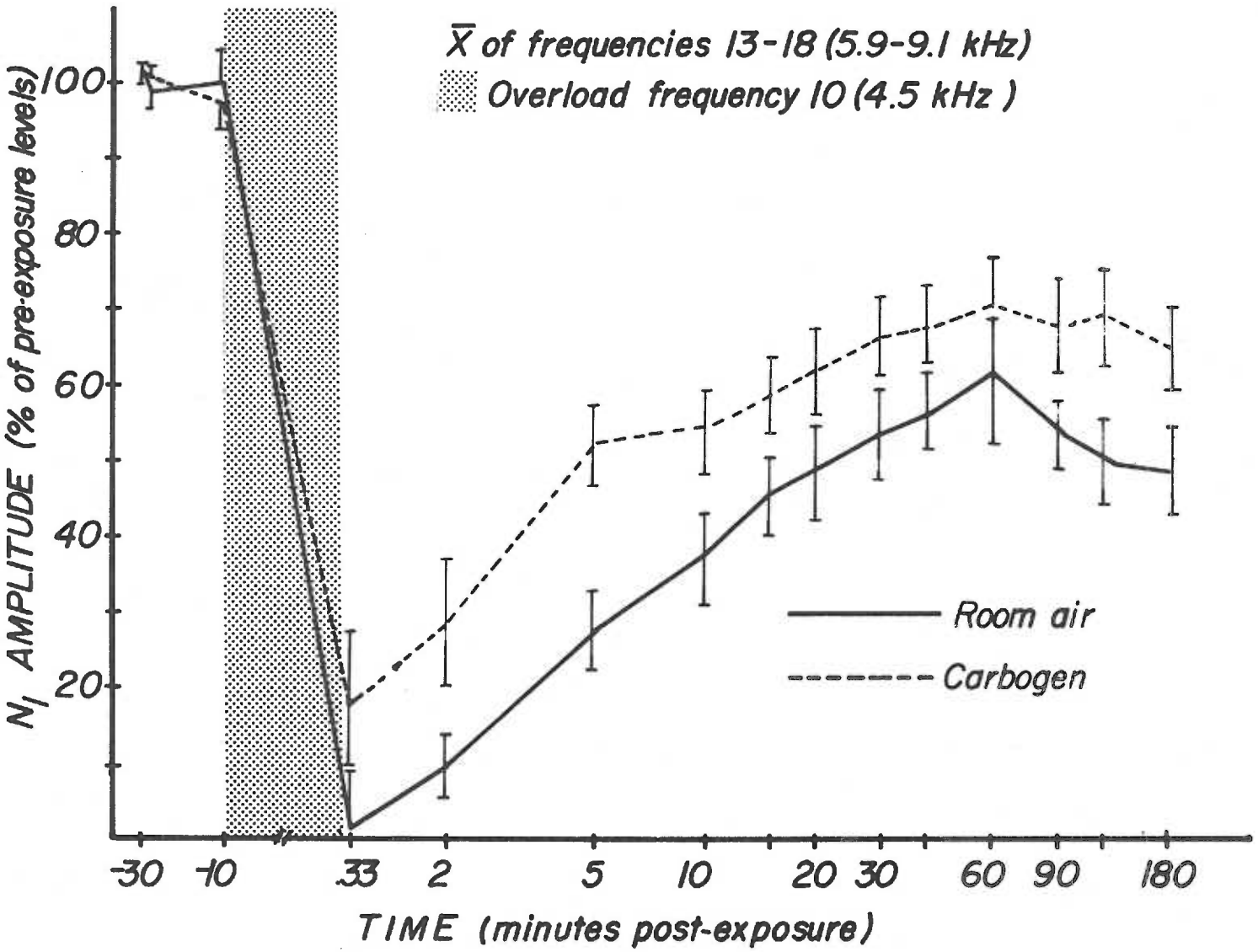

N1 AMPLITUDE (% of pre exposure levels)
100
80
60
40
20
X̄ of frequencies 19-32 (9.9-30 kHz)
Overload frequency 10 (4.5 kHz)
Room air
Carbogen
-30
-10
.33
2
5
10
20
30
60
90
180
TIME (minutes post exposure)

Figure 26. Mean relative recovery (RR) curves obtained from all ears in two groups of animals showing recovery of the N_1 response following an acoustic overload in which N_1 response amplitude is expressed as a percentage value relative to mean pre-exposure levels for each subset of frequencies. Vertical bars represent $\pm$ 1 SEM. Frequencies #13-#18 (5.9-9.1 kHz) are shown.

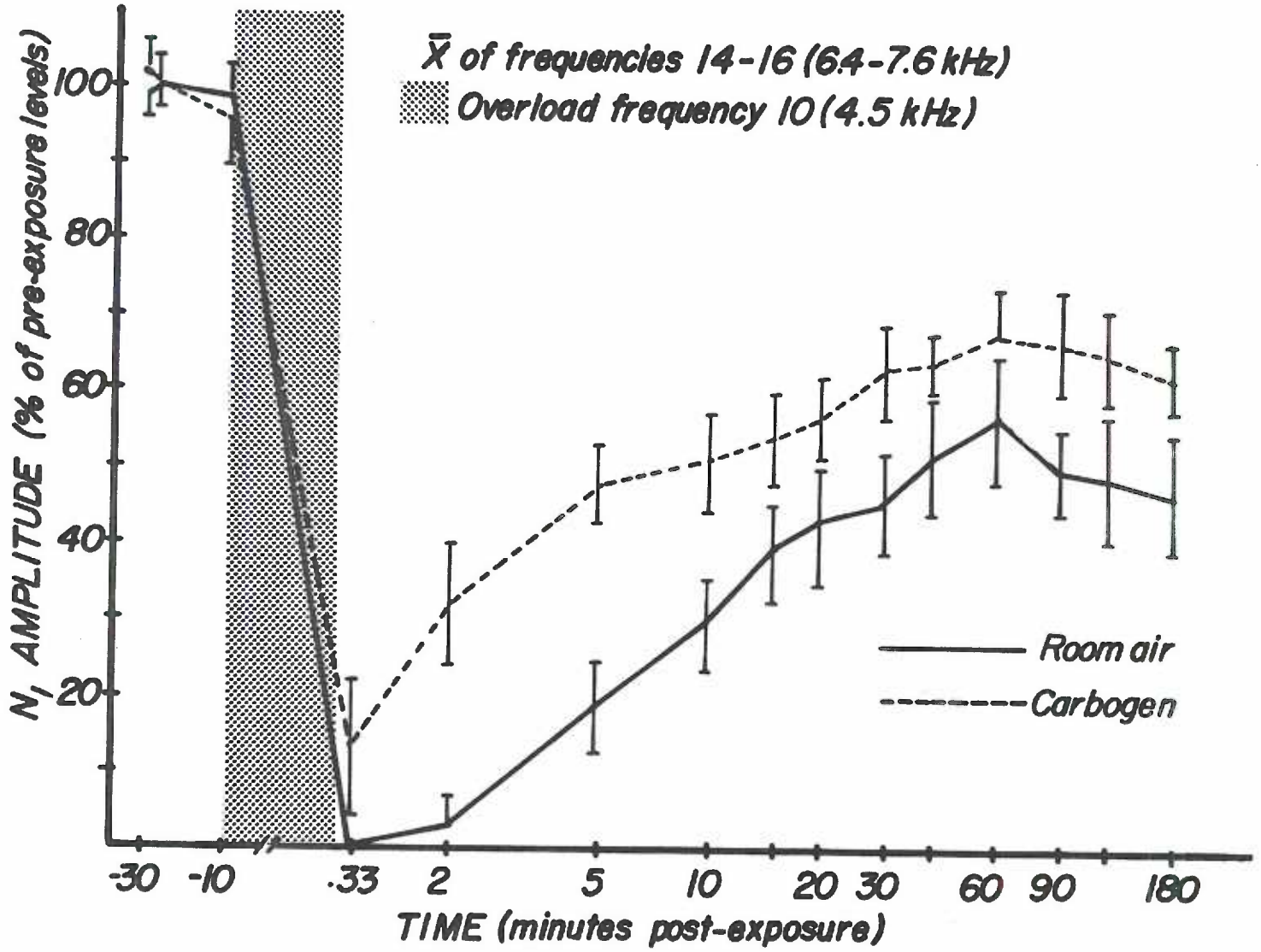

$\bar{X}$ of frequencies 13-18 (5.9-9.1 kHz)
Overload frequency 10 (4.5 kHz)
N_1 AMPLITUDE (% of pre-exposure levels)
100
80
60
40
20
Room air
Carbogen
-30
-10
.33
2
5
10
20
30
60
90
180
TIME (minutes post-exposure)

carbogen showed far less N_1 response depression and more complete recovery throughout the recovery period. To some degree the measures of N_1 suppression in this frequency range are limited by the floor value of 0.0 μV. Maximum recovery of this frequency range occurred in both groups at 60 minutes after the acoustic overload. A slow decline in N_1 response occurred in both groups after this time. After 3 hours of recovery the approximate difference in N_1 response amplitude between the two groups is similar to that in the initial period following overload. This is perhaps due to an approximately equivalent rate of N_1 response recovery in the two groups. This latter observation is based on the finding that the slopes of the recovery curves in both groups are approximately the same.

A more narrow range of frequencies within the area of maximum N_1 depression is shown in Figure 27. This figure demonstrates the mean RR curves for frequencies 14 through 16 (6.4 kHz - 7.6 kHz). Depression and recovery of this set of frequencies shows even more distinct differences between the two experimental groups. Again, the measures in the Room Air group are limited by floor effects. Despite this, the analysis of variance shows these curves to be significantly different ($F_{(1,8)} = 8.05$, $p < .05$). When this figure is compared to the last (frequencies 13-18) it is evident that any increase in N_1 response depression differences between the two groups was found in the first 15 minutes of recovery.

Again, the approximate rate of N_1 response recovery was the

Figure 27. Mean relative recovery (RR) curves obtained from all ears in two groups of animals showing N_1 response recovery following an acoustic overload in which N_1 response amplitude is expressed as a percentage value relative to mean pre-exposure levels for each subset of frequencies. Vertical bars represent ± 1 SEM. Frequencies #14-#16 (6.4-7.6 kHz) are shown.


$\bar{X}$ of frequencies 14-16 (6.4-7.6 kHz)
Overload frequency 10 (4.5 kHz)
N_1 AMPLITUDE (% of pre-exposure levels)
100
80
60
40
20
Room air
Carbogen
-30
-10
.33
2
5
10
20
30
60
90
180
TIME (minutes post-exposure)

same in the two groups and the final level of N_1 response amplitude reflects that immediately following the acoustic exposure. Additionally, the N_1 response declined in both groups after 60 minutes of recovery. The reason for this decline in N_1 response amplitude which occurred in all animals regardless of gas exposure condition was probably due to alterations in blood gas tension which will be discussed below.

DISCUSSION

The results of both experiments confirmed a significant beneficial effect of carbogen inhalation on acoustically-induced auditory impairment in the guinea pig. Experiment I demonstrated a significant reduction in permanent cochlear impairment induced by high intensity broad band noise using the AC cochlear potential and counts of missing inner and outer hair cells as a measure of cochlear function. Experiment II demonstrated significant protection afforded by carbogen inhalation using the degree of N_1 response amplitude depression as a measure of cochlear function. The finding that carbogen inhalation provided protection from acoustically-induced auditory impairment is substantiated not only by the results of each experiment alone, but also by virtue of the fact that similar results were observed in two experiments which employed completely different experimental designs. Further substantiation is provided by the relatively large numbers of animals used in each study. It should be noted that these data confirm and extend the findings of David Lipscomb and his coworkers at the Noise Research Laboratory (48) (108).

Effects of Carbogen on Permanent Cochlear Impairment

The effects of carbogen inhalation on broad band noise-induced permanent cochlear impairment may be summarized as follows: Carbogen inhalation during high intensity acoustic exposure led to a significant decrease in the pathologic elevation of the 1μV isopotential

function of the AC cochlear potential relative to that seen in animals which received only normal air. The beneficial effect was found at all test frequencies but was pronounced at test frequencies below 1.5 kHz. Carbogen inhalation was also found to significantly reduce the number of missing outer hair cells in the cochleae of animals which received it relative to those which did not. Carbogen inhalation alone was not found to lead to any changes in AC cochlear potential or number of missing hair cells in animals which received a very low acoustic exposure relative to those which received normal air.

In all animals exposed to the high intensity noise, the areas of maximum hair cell loss were found within Turn 3, Turn 4 and at the apex of the cochlea. At each site, inhalation of carbogen reduced the magnitude of cell loss. The largest OHC loss was observed within Turn 4 in all animals which received high intensity acoustic exposure regardless of gas inhalation condition.

Statistically significant IHC loss was observed only in the group which received normal air and high intensity sound. There was no difference in the mean loss of IHC's at any site within the cochlea between the group which received carbogen with high intensity sound and the Control Group. There was a consistent relationship between the degree of IHC and OHC damage within each of the two groups which received 120 dB noise. Additionally, there was a consistent ratio between the relative amount of damage to IHC's and that to OHC's between the two groups. In all cases the relative degree of IHC damage was less than that of OHC damage.

A shift in the 1 kHz and in the 10 kHz intensity function of

the AC cochlear potential was observed in the group of animals which received carbogen during acoustic exposure. However, the differences in the mean maximal output of the AC cochlear potential between the two groups which received the high intensity acoustic exposure was not found to be statistically significant.

Arterial blood pH, PO_2 and PCO_2 were substantially altered by inhalation of carbogen during the 4 hours of gas exposure while no changes were observed in these measures in animals which received normal air. After 30 minutes of carbogen inhalation corresponding to initiation of the acoustic overstimulation, the arterial pH declined from a baseline level of 7.38 to 7.28. The arterial PCO_2 showed a corresponding rise from 24 mm Hg to 36 mm Hg and PO_2 rose from 64 mm Hg to 230 mm Hg. These values altered over the remaining 3.5 hours only in that PO_2 appeared to decrease after 60 minutes of gas inhalation and showed an increase after 4 hours in two animals.

The finding that OHC damage was patchy and irregular in the animals which received high intensity acoustic exposure lends morphological evidence to support the proposal that a metabolic type of lesion was produced at the sound levels and durations of exposure used in Experiment I. A mechanically induced lesion resulting from localized basilar membrane displacement would be expected to be present over large contiguous areas of the cochlea with inner hair cell loss in the same location. Loss of IHC's in this experiment was found to be patchy and irregular and bore no precise or consistent relation to areas of OHC loss except in terms of gross location within the cochlea (Turn 3, Turn 4 and apex). The general

degree of IHC loss corresponded with OHC loss from group to group. The inference that this represents a metabolic form of cochlear lesion supports the hypothesis that carbogen inhalation in some way provided sensory cells in the organ of Corti with protection from cellular changes produced by the acoustic exposure. It is conceivable that vascular changes produced by carbogen acted in some way to mechanically reduce the amplitude of displacement of the basilar membrane in response to the acoustic overstimuation. The possibility that carbogen inhalation exerted an effect on the cochlea which effectively decreased the amount of basilar membrane displacement,while unlikely,cannot be ruled out.

It was found that while carbogen inhalation led to a statistically significant reduction of damage measured by the mean 1 μV isopotential function over all test frequencies, this protection was most marked at test frequencies below 1.5 kHz. The finding that OHC loss occurred in all animals which received high intensity acoustic exposure from Turn 3 to the apex of the cochlea corresponds with observed differences in the 1 μV isopotential function between the two groups at different frequencies. Turn 4 in the guinea pig cochlea represents areas of peak basilar membrane displacement due to frequencies from 24 Hz through 1248 Hz according to formulation provided by Greenwood (34). It is within this frequency range that the largest differences in elevation of the isopotential function and of OHC loss were found in the two experimental groups.

It is generally accepted that the AC cochlear potential recorded from the round window membrane reflects primarily the activity

of outer hair cells in or near the basal turns of the cochlea (14) (107). Therefore the question arises as to why any differences in the 1 μV isopotential function were found in groups which showed no differences in OHC loss at the base and within Turn 2 of the cochlea? On the basis of the present data one must assume that the round window recording did in fact represent the activity of cells further toward the apex of the cochlea. The present findings also suggest that damage to hair cells within Turn 3, Turn 4 and at the apex may be reflected throughout the frequency range of the 1 μV isopotential function.

The observation of OHC loss so far apically within the cochlea resulting from exposure to a broad band of noise is somewhat surprising in that this finding is not in precise agreement with previous observations at this laboratory (10). In a study of the effects of broad band noise produced by a single speaker suspended over a wire mesh cage using "free field" conditions, maximum OHC loss was found from Turn 2 through Turn 4 of the guinea pig cochlea (10). Comparison of the 1/3 octave frequency power spectrographs from that and from this study reveals a striking difference. The frequency spectrograph in Experiment I of the present study (Figure 2) shows two distinct maxima. One is a broad area from approximately 900 Hz through 4 kHz. The other maximum is a more narrow sound pressure peak at approximately 250 Hz. It is this latter peak that is absent in the frequency spectrograph from the previous study (10).

It may be suggested that this lower frequency was primarily responsible for the OHC loss observed in animals exposed to the high intensity broad band noise in this study. Spoendlin (92) has shown

OHC losses in Turn 3, Turn 4 and at the apex of the guinea pig cochlea resulting from exposure to a narrow band of noise with a center frequency of 250 Hz at an intensity of 130 dB in a free field situation. It is likely that the peak of sound pressure at this frequency in the present study led to the observed morphological damage. If OHC damage had resulted primarily from higher frequency sound, it would have been predicted to have led to loss of OHC's within the basal turn of the cochlea. Why there was no loss observed within Turn 2 in animals in the present study despite substantial sound pressure at frequencies higher than 250 Hz is not known.

Resonance is increased in any closed chamber relative to free field conditions due to increased sound reflection from surfaces. The peak in the frequency power spectrograph at 250 Hz may correspond to a particular resonance characteristic of the enclosed wooden box used for the acoustic exposure. Resonance of the chamber used in Experiment I would be enhanced by virtue of the fact that the compression drivers which produced the vibration of air were tightly bolted to the wooden enclosure. This probably led to a certain amount of vibration of the wooden box itself which was directly transmitted from the speaker. Although the animals were not in direct contact with the wooden box, further amplification of the resonant frequency may have been provided by close proximity to the wooden wall.

Effects of Carbogen on Arterial Blood Gasses

The observed changes in arterial blood gas values over the 4-hour exposure period and then immediately afterward are relevant to

the recovery of damaged ears in this study. If it is assumed that cochlear blood flow and consequently oxygenation increases directly with arterial PCO_2 (an overly simple assumption) it is evident that cochlear blood flow would reach a maximum very quickly and stay elevated over the remainder of the 3.5 hour acoustic exposure period. The increase in arteriolar vascular diameter would be expected to be compensated for by autoregulatory mechanisms (Bayliss effect) and by sympathetic activation at more proximal arterial sites. Further, during this time the animals would be expected to compensate for respiratory acidosis and hypercarbia by metabolic and respiratory changes. These would include increased reabsorption of bicarbonate ion in the renal tubule and an increase in the rate and depth of respiration. Because the ambient gas itself led to hypercarbia, metabolic compensation would be expected to play the major role in readjustment of body pH. Metabolic compensatory mechanisms are somewhat longer in latency than respiratory mechanism however, and would be expected to persist for some time after carbogen inhalation stopped.

In this regard it is important to note the dramatic fall in PCO_2 and rise in pH which occurred in one animal 30 minutes after cessation of the carbogen inhalation during recovery in room air. This sharp fall to below normal blood gas values was probably the result of a metabolic compensatory mechanism which lagged slightly in time relative to respiratory compensation. By this line of reasoning, the vascular consequences of this compensatory fall in PCO_2 in animals immediately following acoustic exposure would be expected

to have an antagonistic effect to that produced by the gas. Because this would have occurred during a time in which the sensory cells are recovering from the acoustic trauma, this compensation may have played a negative role in recovery from acoustic overload. Such a negative influence would not be expected to occur in animals which received normal air. However by virtue of the evidence that substantial decreases in acoustically-induced auditory impairment were provided by inhalation of carbogen, the negative influence suggested here must be less significant than the positive effects of carbogen inhalation.

Effects of Carbogen on Temporary Cochlear Impairment

The effects of carbogen inhalation on pure-tone induced temporary auditory impairment may be summarized as follows: Inhalation of carbogen during acoustic overload led to a significant decrease in N_1 response depression resulting from a 4.5 kHz pure tone overload at 104 dB. The maximally depressed frequency was found approximately 1/2 octave above the overload frequency in all animals. Carbogen inhalation did not lead to a change in the width of the depressed frequency domain nor did it lead to a shift in the maximally depressed frequency. Inhalation of carbogen during acoustic overload did not lead to substantial changes in the rate of recovery of the maximally depressed frequencies. Differences in rate of recovery from N_1 response depression were observed in frequency domains above and below the maximally depressed frequencies. But in these areas there was far less N_1 response suppression and therefore recovery

appeared to be faster in the groups which received room air. Because the initial degree of N_1 response depression was less at the maximally depressed frequencies in animals which received carbogen during acoustic overload and the relative rate of recovery from N_1 response depression was similar in the two groups, the level of recovery of N_1 response at the end of 3 hours of recovery was higher in the group which received carbogen than in the group which received room air.

The maximally depressed frequency range was similar in width in both groups. The range of frequencies exhibiting some N_1 response depression extended widely on both sides of the maximally depressed frequency. These findings are in agreement with earlier reports of Mitchell (76) and Meikle (69). When various subsets of frequencies were grouped and the degree of depression compared, it was found that the experimental groups differed significantly only in the range of frequencies showing maximal depression. This range extended approximately from 3/8 to 8/8 of an octave above the exposed frequency (5.9 kHz to 9.1 kHz). Above and below this range, there were no significant differences between the two groups over the 3 hours recovery period. It was found that at the very high frequencies (9.9 kHz - 30 kHz) the N_1 responses were more depressed but recovered very quickly in the group which received room air. Similar effects were found in the frequency range below the area of maximal N_1 response depression (2.1 kHz - 5.4 kHz). These effects were only found in the first 5-10 minutes following exposure. Any observed differences in these two frequency domains in terms of N_1 response depression occurred only in the first 10 minutes after the acoustic overload.

Because carbogen inhalation ended at the same time as the acoustic overload it seems probable that the protection or beneficial effects of the gas ended at the same time. This proposal is supported by the finding that although the initial level of damage differed between the two groups, the relative rate of recovery in the maximally depressed frequency range did not.

Carbogen inhalation rapidly altered the arterial pH, PCO_2 and PO_2 in the artificially ventilated animals. It should be remembered that the animals used for blood sampling were respirated using artificial ventilation for approximately 1/2 hour before initial blood samples were drawn. After 20 minutes of carbogen inhalation the pH fell quickly from 7.6 to 7.4, the PCO_2 rose from 20 mm Hg to 33 mm Hg and the PO_2 rose from 87 mm Hg to 140 mm Hg. However, there is a major inconsistency in these data. Comparison of the initial blood gas values (Figure 12) in the animals which were being artificially ventilated with the normal values for the awake and unrestrained guinea pig breathing room air (TABLE IV) shows differences. The net effect of carbogen inhalation in Experiment II apparently was to shift the blood gas and pH values from highly abnormal initial values to more normal blood gas tensions.

The "normal" blood gas values in Experiment I ranged from pH = 7.37 - 7.45; PCO_2 = 24 - 30 mm Hg; and PO_2 = 60 - 70 mm Hg. It is evident that the initial values in Experiment II (taken approximately 1/2 hour after artificial ventilation was initiated) represent guinea pigs which are highly alkalotic, have a substantially decreased PCO_2 and an elevated PO_2. Such effects could be accounted for by over-

ventilation of the animal in the rate or the depth of respiration. A possible consequence of the blood gas values in the anesthetized artificially-ventilated guinea pigs might be a decrease in cochlear blood flow induced by low PCO_2 and high pH.

The procedure which was used to adjust the rate and depth of respiration is presumably at fault. The procedure used was to increase the depth of respiration slowly from low levels until spontaneous middle ear muscle activity (MEMA) ceased. This technique has been used for several years to guage adequacy of respiration at this laboratory (68). It is apparent that in this case the procedure overcompensated for proper levels of respiration. This is most probably due to a failure to adequately compensate for the latency between alteration of respiration depth and appearence of MEMA. Alternatively it may simply require overrespiration of the animal to eliminate MEMA.

Following cessation of the carbogen inhalation there was some compensatory mechanisms evident in terms of adjustment of body pH and PCO_2 but these were not as dramatic as those observed in Experiment I. The compensation in Experiment II did occur at points in time in which recovery was presumed to be taking place.

The findings of extremely low PCO_2 values in the blood of all animals in Experiment II may explain the gradual decline in the N_1 response amplitude that was observed after approximately 60 minutes of recovery from acoustic overload. This point in time corresponds to approximately 2 hours of artificial ventilation for each animal. The decline in N_1 response may be accounted for by a decline in the

functional state of the cochlea in which there was an artificially induced vascular insufficiency due to decreased PCO_2 or increased pH.

Despite the finding that carbogen served to elevate the PCO_2 to normal levels it remains true that this elevation produced a significant beneficial effect. In this regard, the findings of both Experiment I and Experiment II confirm the general hypothesis of this study. That is, that inhalation of a gas which increases blood flow within the cochlea and the oxygenation of that blood led to a decrease in acoustically-induced auditory impairment.

Because such similar effects were observed in two experiments which employed such different designs substantial weight is given to the vascular insufficiency theory of noise induced hearing loss (41)(42)(57)(72). It is evident from these measures that arterial PO_2 increased substantially. Unfortunately the hypothesis that the beneficial effects of carbogen occurred through vascular alterations can only be confirmed through inference. The bulk of experimental evidence (27)(33)(50)(84)(90)(95)(98) however supports this inference.

Other Interpretations

Because it was not possible to directly observe an increase in cochlear blood flow, interpretation of these findings as resulting from such may be premature. There may be other effects of this gas which conceivably participate in the effects observed in these studies (38). Some of these effects of carbogen inhalation include (a) decrease in intracellular pH; (b) changes in plasma HCO_3^-; (c) increase in cardiac output or pulse rate; (d) increased permeability of vessels

due to dilatation and opening of gap junctions or (3) decreased basilar membrane displacement due to some mechanical effect of carbogen on the vessel cells.

Because the general shape, maximally depressed frequencies and rate of recovery of the two groups in Experiment II were very similar, it seems reasonable to eliminate effects of carbogen inhalation on the mechanical performance of the cochlea as a participant in the mechanism of its beneficial effects. A similar inference may be made by virtue of the finding that the magnitude of OHC loss was decreased by inhalation of carbogen during acoustic exposure but no change was observed in the location of OHC loss within the cochlea.

Other of the suggested effects of carbogen may or may not be a participant in the observed findings. When the possible effects of carbogen are evaluated in terms of applicability to the present two studies it may be suggested that, of the direct and indirect effects of carbogen inhalation, none appear more physiologically relevant to the functional state of the cochlea than increased delivery of hyperoxygenated blood. Although assumed through inference rather than observation, it seems safe to present a presumptive hypothesis of the beneficial effects of carbogen inhalation as acting on oxidative metabolism in the acoustically hypoxic organ of Corti.

In terms of the specific aims of these studies we have confirmed that carbogen inhalation during acoustic overexposure leads to reduction in (a) the pathologic permanent elevation of the 1 μV isopotential function of the AC cochlear potential; (b) the number of

missing inner and outer cochlear hair cells 30 days following the insult and (c) the temporary depression of the cochear N_1 response within a specific frequency range. It was inferred that this effect was produced by vasodilation of vessels in the inner ear produced by a measured elevated PCO_2 and increased delivery of oxygen measured as an elevated PO_2. These findings lend strong support to the hypothesis that vascular insufficiency is a large component in some forms of acoustically-induced hearing loss.

A Final Word

The implications of the dependence of cochlear sensory cells on oxygen and on a competent vascular supply which is especially pronounced during acoustic exposure have both basic scientific and clinical implications. Such findings may provide a partial explanation as to why some ears are damaged and some are not by identical acoustical exposures; why there are tough and tender ears. Vascular events may underly instances of sudden deafness in humans and may provide a key to treatment of these disorders. Interruption of oxidative metabolism may play a role in disorders of hearing which appear to have no cause or many causes, such as tinnitus. Lastly, carbogen inhalation may one day provide a therapeutic measure in cases of accidental acoustic trauma. In this instance, treatment usually follows trauma and so the length of time between the initial insult and the application of carbogen must be elucidated in future research.

Future investigations into the effects of acoustic trauma in cochlear oxidative metabolism must also concern itself with understanding the anatomical and biochemical events which may underly the protective effects of carbogen inhalation. Understanding of these mechanisms may allow development of other vasoactive compounds which may provide many of the effects of carbogen inhalation without the inconvenience of gas application.

SUMMARY AND CONCLUSIONS

An investigation was conducted into the possible beneficial effects of inhalation of carbogen gas (95% O_2-5% CO_2) on acoustically-induced permanent and temporary auditory impairment in guinea pigs. Evaluation of permanent cochlear impairment after exposure to high intensity broad band noise was accomplished using the AC cochlear potential and counts of missing inner and outer hair cells from each turn of the cochlea. Evaluation of temporary auditory impairment following a single pure tone sound exposure was accomplished using the amplitude of the N_1 response of the cochlear nerve potential over a large frequency range. In both experiments inhalation of carbogen preceeded and accompanied acoustic overstimulation in one-half of the animals while the other one-half received normal air. It was found that:

1) Carbogen inhalation during acoustic exposure led to a significant protection of the cochlea from the permanent and temporary pathologic effects of intense sound.

2) The general description of the protection afforded by carbogen inhalation was that it reduced the magnitude of cochlear impairment. The morphology and anatomic location of hair cell loss, the range of frequencies affected by the pure tone and the rate of recovery from temporary N_1 response depression were not altered.

Based on the current understanding of the actions of carbogen gas it was concluded that carbogen inhalation led to a reduction in

cochlear damage due to a support of oxidative metabolism in the acoustically-hypoxic organ of Corti. This support was provided by increased delivery of hyperoxygenated blood to the inner ear.

APPENDIX A

Animal Numbers, Group and Weights During the Experimental Period

Tag Number	Weight Day No. 1	Weight Day No. 3	Weight Day No. 5	Weight Day of Sacrifice	Stabilization Interval (Days)
Group A- Compressed Air/50 dB Acoustic Exposure					
10	311	310	305	499	34
35	278	289	300	473	35
99	228	289	300	476	34
100	276	285	296	502	34
no tag	303	320	340	553	33
Group B- Carbogen/50 dB Acoustic Exposure					
2	296	307	340	570	30
22	282	303	340	576	29
56	249	264	295	410	30
70	274	283	310	490	29
94	240	250	280	490	30
Group C- Compressed Air/120 dB Acoustic Exposure					
5	310	319	328	463	32
7	237	250	276	410	34
13	255	268	287	450	34
14	276	274	274	406	34
21	298	303	310	430	32
54	319	329	350	460	35
57	305	318	228	476	32
58	267	276	300	452	34
60	283	293	307	450	37
63	305	325	335	460	34

APPENDIX A (Continued)

Tag Number	Weight Day No. 1	Weight Day No 3	Weight Day No. 5	Weight Day of Sacrifice	Stabilization Interval
Group D- Carbogen/120 dB Acoustic Exposure					
1	250	274	280	424	36
4	268	286	290	486	31
22	205	234	250	391	36
53	234	249	252	366	29
59	325	329	348	451	29
62	324	339	360	478	36
64	215	244	260	390	31
71	230	240	251	360	31
75	300	325	355	510	36
98	310	341	360	453	29

APPENDIX B

Integer Numbering of Frequencies in 1/8 Octave Steps From 1 Through 32 Used in N_1 Recovery Experiment

Test Stimuli

Integer	Actual Frequency (kHz)	Integer	Actual Frequency
1	2.1	26	18.2
2	2.3	27	19.8
3	2.5	28	22.0
4	2.7	29	24.0
5	2.9	30	26.0
6	3.2	31	28.0
7	3.5	32	30.0
8	3.8		
9	4.2		
10	4.5		
11	4.9		
12	5.4		
13	5.9		
14	6.4		
15	7.0		
16	7.6		
17	8.3		
18	9.1		
19	9.9		
20	10.8		
21	11.8		
22	12.8		
23	14.0		
24	15.3		
25	16.6		

APPENDIX C

ANALYSIS OF VARIANCE FOR 1 μv ISOPOTENTIAL FUNCTIONS

(One way, equal group numbers)

A. Over all test frequencies:

2105.0653 = SSB
923.3620 = SSE
3028.4273 = SST
1052.5326 = A
16.1993 = B
64.9738 = F-Value, $p < .001$
2.0000 = k-1
57.0000 = k(n-1)

GROUPS INVOLVED	SOURCE OF VARIATION	F	DF	p
Control X Carbogen/120	Treatment	41.5	(2,57)	$< .001$
Control X Compressed Air/120	Treatment	98.5	(2,57)	$< .001$
Carbogen/120 X Compressed Air/120	Treatment	12.12	(2,57)	$< .01$

B. Over test frequencies 100 Hz- 1.5 kHz:

1573.4333 = SSB
1006.7500 = SSE
2580.1833 = SST
786.7166 = A
17.6622 = B
44.5421 = F-Value, $p < .001$
2.0000 = k-1
57.0000 = k(n-1)

GROUPS INVOLVED	SOURCE OF VARIATION	F	DF	p
Control X Carbogen/120	Treatment	32.7	(2,57)	$< .001$
Control X Compressed Air/120	Treatmant	88.4	(2,57)	$< .001$
Carbogen/120 X Compressed Air/120	Treatment	13.59	(2,57)	$< .005$

APPENDIX C (Continued)

C. Over test frequencies 2kHz- 20 kHz:

1747.4333 = SSB
1158.7500 = SSE
2906.1833 = SST
873.7166 = A
20.3289 = B
42.9789 = F-Value, $p < .001$
2.0000 = k-1
57.0000 = k(n-1)

GROUPS INVOLVED	SOURCE OF VARIATION	F	DF	p
Control X Carbogen/120	Treatment	41.6	(2,57)	$< .001$
Control X Compressed Air/120	Treatment	81.89	(2,57)	$< .001$
Carbogen/120 X Compressed Air/120	Treatment	6.73	(2,57)	$< .05$

APPENDIX D

ANALYSIS OF VARIANCE FOR INTENSITY FUNCTIONS

(One-way, equal group numbers)

A. 1 kHz:

5061338.8000 = SSB
3185384.8000 = SSE
8246723.6000 = SST
2530669.4000 = A
55883.9438 = B
45.2843 = F Value, $p < .001$

GROUPS INVOLVED	SOURCE OF VARIATION	F	DF	P
Control X Carbogen/120	Treatment	59.3	(2,57)	$< .001$
Control X Comppessed Air/120	Treatment	75.3	(2,57)	$< .001$
Carbogen/120 X Compressed Air/120	Treatment	.95	(2,57)	ns

B. 10 kHz

59868.1000 = SSB
392338.3000 = SSE
452206.4000 = SST
29934.0500 = A
6883.1280 = B
4.3489 = F-Value, $p < 05$
2.0000 = k-1
57.0000 = k(n-1)

GROUPS INVOLVED	SOURCE OF VARIATION	F	DF	p
Control X Carbogen/120	Treatment	.52	(2,57)	ns
Control X Compressed Air/120	Treatment	8.17	(2,57)	$< .05$
Carbogen/120 X Compressed Air/120	Treatment	4.55	(2,57)	ns

APPENDIX E

Complete listing of counts of missing OHC's from 3 groups of animals in Experiment I. Values are expressed as percentages relative to the total number that would be present in equivalent segments of perfect organ of Corti.

Compressed Air/120 Group

Animal No.	Apex	Turn 4	Turn 3	Turn 2	Base
63-L		67	21	6	2
R	100	27	12	6	0
14-L	18	33	14	6	5
R	12	19	10	14	2
50-L	19	21	11	5	3
R	19	21	12	5	1
54-L		30	26	5	0
R	20	21	35	1	3
5-L	9	18	25	6	0
R	9	27	30	9	2
13-L	14	26	11	4	0
R	12	9	10	5	0
58-L	22	32	23	6	0
R	32	44	17	3	1
21-L	33	100	83	8	6
R	66	25	33	0	0
57-L	19	27	19	0	0
R		9	15	3	0
76-L	25	35	9	1	0
R	64	30	10	0	0
X	29	31	21	5	1.25
SD	23	20	17	3.4	1.8
SEM	5.2	4.6	3.8	0.78	0.4

Carbogen/120 Group

Animal No.	Apex	Turn 4	Turn 3	Turn 2	Base
22-L	15	8	8	13	0
R	15	12	9	7	0
98-L	6	5	0	1	0
R	14	7	3	1	0
59-L	21	16	10	0	0
R	18	15	6	1	0
4-L	3	3	3	3	0
R	4	8	3	0	0
75-L	9	8	4	1	0
R	8	4	4	1	0
1-L	5	8	17	1	0
R	5	37	19	2	0
53-L	6	9	1	0	0
R	6	13	3	8	1
71-L	13	20	24	1	0
R	7	10	9	6	0
64-L	10	17	12	4	0
R		26	6	0	0
62-L	17	14	3	1	1
R	17	19	2	1	0
X	10.4	13	7.3	2.1	0.1
SD	5.3	7	6.4	3.4	0.0
SEM	1.2	1.8	1.4	0.78	0.0

Control Group

0-L	6	0	0	0	0
R	5	3	0	1	0
99-L	8	0	0	0	1
R	6	5	0	1	0
10-L		3	0	0	0
R	5	6	1	0	0
35-L	7	2	1	0	0
R	12	4	1	1	0
100-L	7	3	0	2	0
R		4	1	0	0
2-L	5	2	2	0	0
R	6	5	1	0	0
70-L	6	2	0	0	1
R	1	1	0	0	0
56-L	8	8	0	0	0
R	7	6	0	0	0
22-L	4	3	1	0	0
R	5	2	1	0	0
94-L	6	0	0	0	0
R	6	5	2	0	0
X	6.1	3.2	0.55	0.25	0.1
SD	2.0	2.2	0.6	0.5	0.0
SEM	0.4	0.5	0.1	0.1	0.0

APPENDIX F

ANALYSIS OF VARIANCE FOR OUTER HAIR CELL COUNTS

(2-way, repeated measures on B, location)

	SS	DF	MS	F	P
BET SUBJECTS	20542.986	59			
A	12412.586	2	6206.293	43.51	< .001
ERROR	8130.400	57	142.638		
WITH SUBJECTS	36389.200	240			
B	11991.886	4	2997.971	37.51	< .001
AB	6177.813	8	772.226	9.66	
ERROR	18219.500	228	79.910		

GROUP	SOURCE OF VARIATION	DF	F	p
Control X Carbogen/120	Treatment	(2,57)	4.52	< .05
Control X Compressed Air/120	Treatment	(2,57)	49.6	< .001
Carbogen/120 X Compressed Air/120	Treatment	(2,57)	24.16	< .001
Above at APEX	Treatment	(1,18)	24.12	< .001
Above at T_4	Treatment	(1,18)	22.96	< .001
Above at T_3	Treatment	(1,18)	13.7	< .001
Above at T_2	Treatment	(1,18)	.28	ns
Above at BASE	Treatment	(1,18)	.05	ns

APPENDIX G

Complete listing of counts of missing IHC's from 3 groups of animals in Experiment I. Values are expressed as percentages relative to the total number that would be present in equivalent segments of perfect organ of Corti.

Compressed Air/120 Group

Animal No.	Apex	Turn 4	Turn 3	Turn 2	Base
63-R	10	5	3	0	0
L		5	5	0	0
14-R	5	10	6	3	0
L	4	6	3	3	0
50-R	4	5	3	2	0
L	14	3	0	0	0
54-R	6	2	6	0	0
L		1	3	0	0
5-R	5	7	6	0	0
L	8	6	9	4	0
13-R	5	2	0	0	0
L	3	6	10	2	0
58-R	16	8	8	2	0
L	25	16	5	0	0
21-R	20	30	10	10	0
L	12	9	7	1	0
57-R		20	5	1	0
L	4	5	5	2	0
76-R	20	8	3	2	0
L	11	13	3	0	0
X	9.5	8.4	5	1.6	0
SD	6.5	6.9	2.8	2.3	0
SEM	1.5	1.6	.65	.5	0

Carbogen/120 Group

22-R	4	2	2	0	0
L	3	2	0	2	0
98-R	0	0	0	0	0
L	2	0	0	0	0
59-R	4	3	3	0	0
L	7	7	5	0	0
4-R	2	1	6	1	0
L	2	4	0	0	0

Appendix G, Continued)

Animal No.	Apex	Turn 4	Turn 3	Turn 2	Base
75-R	2	3	1	0	0
L	2	4	0	0	0
1-R	4	3	3	0	0
L	3	8	3	0	0
53-R	4	3	0	0	0
L	3	4	2	0	0
71-R	5	4	2	0	0
L	3	4	2	0	0
64-R		0	0	0	0
L	3	3	1	0	
62-R	4	4	5	0	0
L	4	6	2	0	0
X	3.15	3.2	1.8	0.15	0.0
SD	1.4	2.1	1.8	0.49	0.0
SEM	0.33	0.5	0.43	0.11	0.0

Control Group

0-R	2	1	0	0	0
L	3	2	0	0	0
99-R	2	0	0	0	0
L	3	0	0	0	0
10-R	4	5	3	1	0
L		4	1	0	0
35-R	5	4	0	0	0
L	3	0	0	0	0
100-R		3	0	0	0
L	3	2	0	0	0
2-R	5	3	2	0	0
L	3	3	2	0	0
70-R	5	4	2	0	0
L	2	0	0	0	0
56-R	5	3	0	0	0
L	8	2	0	0	0
22-R	3	2	0	0	0
L	1	2	0	0	0
94-R	2	2	0	0	0
L	3	0	0	0	0
X	3.5	2.1	0.5	0.0	0.0
SD	1.6	1.5	0.0	0.0	0.0
SEM	0.36	0.55	0.0	0.0	0.0

APPENDIX H

ANALYSIS OF VARIANCE FOR INNER HAIR CELL COUNTS

(2-way, repeated measures on B, location)

	SS	DF	MS	F	P
BET SUBJ	1700.596	59			
A	797.726	2	398.863	25.8	<.001
Error	902.870	57	15.839		
WITH SUBJ	3131.600	240			
B	1345.813	4	336.453		
AB	398.106	8	49.763	55.28	<.001
Error	1387.680	228	6.06	8.17	

Group	Source of Variation	DF	F	P
Control x Carbogen/120	Treatment	(2,57)	0.33	ns
Control x Compressed Air/120	Treatment	(2,57)	25.2	<.001
Carbogen/120 x Compressed Air/120	Treatment	(2,57)	19.78	<.001
Above at Apex	Treatment	(1,18)	199.8	<.001
Above at Turn 4	Treatment	(1,18)	131.4	<.001
Above at Turn 3	Treatment	(1,18)	50.7	<.001
Above at Turn 2	Treatment	(1,18)	10.4	<.05
Above at Base	Treatment	(1,18)	0.0	ns

APPENDIX I

Averaged N_1 response amplitudes at 32 frequencies in 15 Test Sequences expressed as percentage values relative to mean N_1 response amplitude in Test Sequence 1 through 3.

G.P. #1 (Room Air)

FREQ	TS1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 %	121	178	0	172	151	130	121	95	169	109	98	172	145	83	71
2 %	36	141	121	103	93	82	121	98	89	77	148	114	116	82	77
3 %	159	38	102	75	65	86	88	102	72	150	68	63	86	68	113
4 %	65	127	107	88	77	72	100	82	77	60	84	102	47	72	79
5 %	138	104	56	121	106	88	100	102	100	125	106	134	121	56	52
6 %	110	96	92	92	84	86	79	81	83	86	65	66	48	78	71
7 %	83	133	83	88	78	138	46	70	151	86	125	148	140	75	83
8 %	105	86	107	61	53	44	52	73	62	66	73	55	35	68	89
9 %	95	97	106	55	48	34	57	76	82	70	110	68	91	57	57
10 %	75	128	96	57	53	51	64	68	87	42	90	77	77	83	68
11 %	106	96	97	39	36	49	48	55	58	61	65	48	39	58	55
12 %	108	110	81	32	30	45	46	46	52	63	56	61	52	50	28
13 %	100	116	82	.0	0	37	23	51	43	39	73	73	51	39	49
14 %	111	86	102	0	0	0	14	43	26	55	46	43	39	50	53
15 %	79	118	101	0	0	21	21	31	44	50	60	68	48	64	50
16 %	98	112	89	21	0	51	28	53	63	46	77	96	60	72	72
17 %	94	88	117	5	43	51	55	69	44	73	48	67	42	55	80
18 %	103	104	92	43	52	65	48	54	88	70	66	83	68	65	61
19 %	97	89	113	49	51	78	58	53	70	78	84	73	80	75	73
20 %	95	103	100	40	50	56	60	56	53	72	58	50	48	51	75
21 %	108	93	98	50	67	68	63	86	68	75	63	63	67	79	75
22 %	118	99	81	74	79	81	91	87	72	105	89	72	83	75	79
23 %	120	109	69	76	73	99	80	85	92	90	88	75	90	62	62
24 %	95	112	92	68	72	75	61	74	86	72	79	77	83	66	52
25 %	98	98	103	52	76	62	72	90	66	72	80	45	76	80	82
26 %	107	97	95	79	81	75	70	79	97	62	75	85	60	75	79
27 %	112	100	87	52	83	81	81	92	89	89	87	69	65	58	77
28 %	99	108	92	83	88	92	102	92	92	95	99	102	94	85	88
29 %	105	103	90	59	75	82	78	86	92	86	80	88	86	71	78
30 %	105	103	90	58	67	76	85	88	86	75	80	84	82	75	76
31 %	104	98	96	30	49	74	87	83	89	96	89	87	91	66	77
32 %	103	99	97	36	52	66	81	97	83	87	87	95	79	81	77

APPENDIX I (continued)

G.P. #2 (Carbogen)

FREQ		TS1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	%	94	89	116	79	75	58	77	60	79	61	32	58	54	54	56
2	%	114	105	79	79	77	67	84	60	79	58	65	65	53	53	60
3	%	82	122	95	95	79	82	76	63	74	66	82	63	53	53	47
4	%	97	119	83	100	102	102	75	83	58	58	102	69	61	50	55
5	%	114	97	88	80	76	71	78	71	69	66	69	73	47	59	59
6	%	96	133	70	84	98	89	67	89	53	77	67	58	63	53	65
7	%	112	67	120	63	63	54	74	44	74	61	33	74	49	63	61
8	%	88	125	86	86	90	101	72	94	52	61	88	50	39	50	48
9	%	100	102	96	75	73	61	65	63	50	56	46	46	52	40	44
10	%	118	89	92	40	80	61	70	68	51	47	47	49	44	44	44
11	%	94	129	75	49	59	75	61	68	33	33	75	33	30	40	33
12	%	112	116	70	25	34	43	41	45	32	41	43	41	27	34	43
13	%	125	87	87	8	26	26	30	24	40	42	20	48	40	50	52
14	%	102	147	50	12	30	40	30	42	50	35	72	45	42	42	55
15	%	105	101	93	16	28	32	28	32	42	44	38	52	44	44	50
16	%	115	86	98	16	30	37	43	37	45	45	39	54	47	49	50
17	%	100	135	64	35	50	61	45	61	40	57	69	57	57	52	57
18	%	108	94	97	41	50	55	57	57	63	52	52	68	48	66	48
19	%	115	82	101	39	54	54	64	49	64	60	64	70	54	68	58
20	%	91	120	88	51	57	53	49	47	51	60	62	51	53	53	64
21	%	100	119	80	59	61	55	51	44	53	61	63	63	61	55	65
22	%	111	113	75	57	62	55	42	40	51	53	62	66	53	66	62
23	%	106	100	93	56	68	45	27	29	41	41	56	54	50	60	56
24	%	102	97	100	66	58	41	25	29	33	37	41	45	45	52	58
25	%	108	105	86	72	64	33	31	21	33	33	50	40	40	50	57
26	%	107	97	95	71	69	33	21	19	45	33	42	47	54	57	64
27	%	106	117	76	81	68	38	24	27	46	51	54	57	57	57	73
28	%	106	106	86	66	63	53	27	43	48	55	66	63	63	63	83
29	%	105	100	94	69	73	58	53	51	58	64	73	69	62	64	71
30	%	110	98	91	50	62	55	52	52	60	62	67	64	64	67	64
31	%	103	106	89	47	47	61	66	51	66	63	66	70	63	61	59
32	%	102	107	90	40	52	60	62	67	57	67	62	67	65	57	62

APPENDIX I (continued)

G.P. #3 (Room Air)

FREQ	TS1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 %	104	104	91	60	35	89	39	63	79	36	89	92	50	60	51
2 %	110	107	82	70	63	97	66	63	93	68	80	78	57	64	55
3 %	96	79	123	102	132	81	127	100	81	94	81	81	79	67	46
4 %	74	97	128	137	117	105	162	117	82	154	85	120	117	65	65
5 %	107	100	92	77	89	114	80	80	85	54	101	64	71	64	42
6 %	80	102	117	100	93	57	95	104	57	100	63	87	106	61	65
7 %	110	119	69	31	27	93	41	62	97	48	111	66	48	66	54
8 %	96	91	111	80	94	69	111	98	67	129	51	80	107	49	55
9 %	98	94	107	32	37	71	41	92	86	81	81	96	64	58	62
10 %	103	101	95	20	34	75	58	68	84	71	86	79	75	60	57
11 %	100	88	111	26	57	66	115	91	77	117	117	88	100	60	60
12 %	110	92	97	0	21	51	64	56	82	86	94	77	82	60	47
13 %	111	111	76	0	0	28	29	43	54	39	75	69	53	42	45
14 %	91	104	104	0	0	8	37	45	37	54	61	65	58	33	35
15 %	111	107	81	0	0	8	26	31	33	44	58	58	41	29	38
16 %	110	110	80	0	0	21	30	36	46	36	66	60	41	40	33
17 %	85	98	116	0	0	15	41	41	37	76	43	48	72	26	41
18 %	122	106	71	0	11	46	57	51	66	60	93	64	44	55	44
19 %	119	95	85	15	33	61	65	51	83	63	109	71	47	67	49
20 %	83	108	108	0	47	33	87	89	75	100	54	79	95	41	52
21 %	84	103	111	49	76	37	103	86	76	109	37	80	92	39	58
22 %	96	94	108	47	52	64	67	96	59	88	59	74	62	47	54
23 %	122	98	78	54	80	91	89	85	91	74	121	76	78	72	65
24 %	98	112	88	43	53	83	53	73	87	62	75	82	51	58	50
25 %	103	96	100	59	75	53	92	73	73	98	71	92	75	44	44
26 %	110	97	92	53	71	97	105	79	92	107	114	84	75	60	51
27 %	90	102	108	50	68	84	86	98	78	98	84	74	92	62	42
28 %	108	93	97	48	69	91	95	93	106	95	104	95	83	67	59
29 %	93	99	107	53	70	99	101	107	107	103	103	91	78	57	51
30 %	95	110	93	55	76	91	100	87	95	91	93	89	78	61	53
31 %	100	96	102	39	50	77	98	96	92	86	102	88	67	56	54
32 %	101	90	108	28	50	79	88	99	88	94	97	81	68	55	48

APPENDIX I (continued)

G.P. #4 (Carbogen)

FREQ	TS1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 %	102	98	98	65	83	48	64	25	65	75	45	34	67	39	37
2 %	100	106	93	115	115	79	81	43	72	75	86	70	68	52	59
3 %	105	102	92	75	92	125	97	47	70	102	95	100	77	97	62
4 %	104	90	104	99	107	110	107	57	74	90	115	129	77	96	63
5 %	111	102	85	66	111	73	69	47	100	119	76	88	76	80	57
6 %	110	100	89	78	91	110	110	47	67	91	102	89	78	78	45
7 %	108	101	90	88	101	36	47	26	101	69	33	27	64	48	38
8 %	93	96	110	82	82	130	118	73	62	90	138	121	67	84	65
9 %	97	97	105	69	88	67	97	33	76	101	80	69	80	69	63
10 %	96	101	101	65	78	43	56	27	60	70	74	47	74	50	50
11 %	102	97	100	55	72	120	75	85	65	92	117	115	90	112	50
12 %	102	112	85	36	78	100	68	80	75	107	109	90	70	95	53
13 %	102	107	89	40	61	47	58	22	66	66	58	40	68	53	40
14 %	95	102	102	41	46	86	72	79	36	76	100	91	69	86	55
15 %	105	111	83	42	59	56	61	42	56	69	58	54	81	58	56
16 %	95	98	105	51	78	41	55	39	71	76	49	68	81	43	44
17 %	92	106	101	48	52	103	59	75	52	74	88	84	63	84	57
18 %	110	92	96	69	113	80	71	78	84	80	84	76	63	71	51
19 %	108	86	106	72	60	88	84	66	80	92	88	74	70	76	44
20 %	86	112	101	76	76	116	97	116	56	97	103	110	90	99	75
21 %	99	97	103	80	37	114	97	116	54	93	116	105	78	105	58
22 %	98	96	104	92	84	78	92	67	76	92	88	104	94	80	59
23 %	106	97	95	79	81	89	77	87	87	95	89	77	87	83	60
24 %	93	99	107	91	99	85	75	66	81	81	81	74	74	72	64
25 %	100	95	104	93	85	95	77	85	64	87	93	102	93	95	60
26 %	104	100	95	100	127	88	67	67	76	90	97	88	97	83	69
27 %	97	97	105	90	92	99	81	90	61	90	97	105	88	88	59
28 %	94	94	111	79	105	77	66	72	68	77	75	79	87	87	57
29 %	100	98	100	67	94	80	69	73	67	80	76	88	90	84	57
30 %	102	92	104	73	78	78	71	65	71	73	84	78	69	69	59
31 %	97	109	93	68	78	78	76	78	76	86	84	88	80	74	60
32 %	101	106	92	56	74	101	72	74	72	81	87	87	96	81	56

APPENDIX I (continued)

G.P. #6 (Carbogen)

FREQ	TS1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 %	103	90	105	120	108	142	125	135	127	100	118	73	88	88	90
2 %	96	70	132	132	144	155	115	132	127	108	132	125	129	125	118
3 %	107	109	82	84	105	120	103	84	80	130	82	93	95	82	72
4 %	73	88	138	108	106	101	90	93	105	80	108	93	91	88	84
5 %	115	92	92	100	98	125	111	113	102	106	98	98	92	96	104
6 %	97	113	89	101	115	95	93	107	97	111	103	109	117	117	111
7 %	97	103	99	86	103	141	122	128	118	101	103	111	86	78	69
8 %	97	95	106	81	99	101	88	90	93	103	103	95	83	83	77
9 %	89	111	99	81	97	123	107	105	109	105	105	99	109	101	93
10 %	89	96	114	51	74	97	86	92	91	86	97	94	77	77	76
11 %	101	94	103	36	65	87	74	81	82	93	98	82	81	82	82
12 %	93	100	105	15	49	63	56	64	68	75	80	75	76	76	76
13 %	94	88	116	0	22	45	40	48	55	52	62	62	55	59	64
14 %	93	92	113	6	16	47	41	44	51	56	56	57	56	57	59
15 %	91	91	116	0	21	51	45	45	54	61	72	74	70	70	70
16 %	91	95	113	0	20	54	47	54	58	67	75	71	76	76	76
17 %	98	93	107	8	32	59	51	54	61	69	76	75	73	76	80
18 %	87	98	114	12	40	72	62	67	67	79	79	80	85	85	83
19 %	99	93	106	23	46	81	68	75	83	90	99	97	95	95	95
20 %	89	95	115	34	65	90	75	79	82	89	98	102	95	93	90
21 %	88	88	122	60	88	98	82	85	90	98	109	104	100	100	98
22 %	91	95	113	70	91	108	89	92	97	105	116	114	105	100	94
23 %	91	95	113	80	106	122	102	102	109	120	131	126	115	113	107
24 %	85	93	120	89	107	120	100	100	102	120	128	128	115	104	95
25 %	97	91	111	100	121	125	105	109	109	125	135	128	116	105	100
26 %	95	95	109	93	116	129	107	107	109	111	123	122	111	104	97
27 %	98	92	108	89	110	130	107	108	108	125	125	114	110	107	101
28 %	92	96	111	76	100	103	111	115	121	121	125	121	109	105	101
29 %	98	90	110	55	73	90	96	104	108	106	114	106	93	92	86
30 %	93	103	103	47	64	82	101	103	108	114	119	115	114	108	103
31 %	99	105	95	35	49	71	87	107	109	115	123	115	117	117	115
32 %	100	95	104	33	53	60	97	111	113	122	122	111	115	111	106

APPENDIX I (continued)

G.P. #8 (Room Air)

FREQ	TS1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 %	91	118	89	102	89	102	124	132	124	140	108	113	89	105	75
2 %	78	121	100	82	121	115	108	92	98	94	90	100	90	98	88
3 %	96	118	85	94	102	98	129	100	107	96	122	116	96	100	98
4 %	95	117	87	100	73	139	100	95	107	121	117	97	92	107	82
5 %	84	95	119	92	92	138	130	143	116	153	114	108	127	143	116
6 %	95	93	111	77	106	111	93	111	102	108	93	86	113	122	93
7 %	86	106	106	0	95	63	98	150	86	147	112	129	118	124	86
8 %	93	98	108	59	95	87	100	103	98	111	116	113	126	106	106
9 %	113	85	101	48	64	66	99	99	94	94	94	103	92	85	78
10 %	91	94	114	37	79	86	114	96	116	94	94	114	104	91	101
11 %	95	110	93	0	41	56	49	54	66	59	73	66	76	56	71
12 %	103	101	94	0	9	32	23	36	39	53	48	46	55	50	43
13 %	84	96	120	0	0	24	45	60	50	43	55	55	57	50	40
14 %	105	93	100	0	0	13	29	41	45	52	52	54	43	48	45
15 %	100	93	105	.0	0	16	45	50	52	48	40	57	57	57	55
16 %	106	82	111	0	0	0	31	46	46	56	51	56	75	72	56
17 %	104	99	96	0	0	31	39	44	52	67	65	73	75	57	60
18 %	94	96	108	0	33	33	51	51	54	73	68	70	61	68	59
19 %	57	95	147	0	31	49	63	54	77	86	89	98	112	95	98
20 %	104	111	83	40	47	66	59	80	64	85	88	83	90	92	88
21 %	87	107	105	43	66	84	82	92	89	105	94	102	105	105	84
22 %	99	87	113	51	77	87	99	92	103	101	99	106	106	101	96
23 %	98	103	98	57	72	84	98	105	96	110	93	96	100	103	86
24 %	89	100	110	56	94	89	94	91	108	83	110	121	124	118	113
25 %	94	99	106	55	73	86	83	96	104	101	111	86	101	94	88
26 %	108	103	87	67	77	95	95	98	95	98	108	108	95	95	85
27 %	90	100	109	54	81	81	117	111	117	122	95	117	119	133	106
28 %	102	91	105	65	80	85	102	108	88	128	108	114	105	102	100
29 %	100	98	100	57	77	92	100	98	98	115	112	112	106	106	95
30 %	86	100	113	62	96	89	82	93	106	96	120	117	124	124	117
31 %	100	93	106	71	68	78	75	93	118	100	112	115	118	121	109
32 %	91	109	98	62	87	80	91	120	91	102	109	113	120	113	109

APPENDIX I (continued)

G.P. #9 (Carbogen)

FREQ	TS1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 %	100	106	92	71	103	98	145	86	115	100	139	109	175	118	148
2 %	80	132	87	163	205	198	139	202	139	132	205	212	209	181	108
3 %	108	90	101	96	87	110	66	80	152	120	83	76	115	94	126
4 %	105	88	105	83	95	95	95	140	132	115	115	113	142	118	110
5 %	60	123	116	101	161	148	132	176	142	97	189	208	195	126	101
6 %	50	207	42	242	257	275	153	292	160	64	214	275	278	207	114
7 %	103	63	132	27	50	52	106	68	95	124	52	67	32	85	116
8 %	116	88	94	48	59	79	87	101	100	114	75	83	90	118	98
9 %	107	83	109	50	55	90	113	81	93	116	78	81	100	95	102
10 %	109	73	116	19	50	59	89	73	100	116	88	86	62	98	84
11 %	83	116	100	30	70	85	91	116	122	100	94	113	135	124	107
12 %	113	105	80	12	39	47	95	91	80	91	82	101	99	72	68
13 %	85	107	107	0	15	47	72	74	66	76	64	72	78	80	70
14 %	107	93	99	0	23	49	73	75	79	75	57	81	83	71	67
15 %	103	105	90	0	21	40	53	69	67	65	61	73	71	75	65
16 %	103	96	100	0	20	55	61	70	62	70	70	81	75	83	68
17 %	113	84	101	15	36	51	65	78	78	78	80	90	71	84	94
18 %	91	110	97	0	38	53	61	75	77	81	77	85	102	91	93
19 %	97	91	110	8	42	68	70	89	93	82	93	102	89	100	91
20 %	93	101	105	28	60	62	83	99	93	97	101	111	103	101	87
21 %	98	96	105	57	64	94	94	107	100	92	105	103	105	96	111
22 %	101	114	83	58	87	92	94	101	101	110	89	108	105	108	101
23 %	94	104	100	66	75	91	106	93	110	100	113	102	108	108	110
24 %	92	106	101	90	92	108	113	120	106	117	110	124	110	113	113
25 %	95	102	102	75	68	84	95	97	82	97	102	97	95	91	100
26 %	101	74	124	93	95	80	88	91	86	99	84	93	88	84	84
27 %	80	95	124	95	86	65	82	90	97	84	105	95	105	99	92
28 %	82	103	114	106	100	79	103	84	103	103	98	87	95	106	79
29 %	88	100	111	66	73	71	75	88	88	82	75	66	55	57	48
30 %	100	93	106	48	63	80	91	85	100	104	95	100	102	85	70
31 %	98	98	102	46	65	75	83	90	92	98	92	104	94	92	67
32 %	100	97	102	52	67	65	80	102	95	95	97	92	102	90	60

APPENDIX I (continued)

G.P. #10 (Carbogen)

FREQ	TS1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 %	99	94	106	91	89	91	71	106	94	99	86	104	91	81	79
2 %	151	66	82	102	120	107	115	53	110	135	58	164	128	143	158
3 %	110	105	84	117	128	105	128	92	148	110	141	117	102	143	112
4 %	122	80	97	117	134	145	122	102	140	125	94	137	131	120	125
5 %	122	92	84	127	122	119	106	92	106	151	66	156	151	119	130
6 %	131	76	92	30	84	30	88	86	73	141	41	156	129	110	129
7 %	80	123	95	139	123	170	136	132	148	71	228	61	46	71	86
8 %	87	97	115	49	59	71	63	103	73	81	115	77	71	69	67
9 %	83	107	109	50	64	77	68	119	93	75	117	75	56	68	81
10 %	98	105	96	47	62	96	105	83	111	60	120	83	70	81	87
11 %	102	113	84	30	67	78	91	82	108	95	89	110	113	113	102
12 %	136	82	80	10	39	67	67	80	78	104	104	121	110	110	113
13 %	108	89	101	0	19	35	55	60	62	71	75	83	75	71	66
14 %	102	98	98	9	25	39	45	57	71	59	84	67	80	76	73
15 %	129	87	83	0	30	43	56	55	58	75	49	81	68	75	81
16 %	103	99	97	5	33	45	53	71	65	71	79	69	69	65	59
17 %	109	105	84	20	52	68	87	68	84	61	84	91	66	75	84
18 %	88	109	101	21	44	53	59	75	71	84	76	73	86	80	73
19 %	116	85	97	24	47	64	72	59	76	64	78	95	84	95	107
20 %	121	89	89	41	62	72	70	70	77	98	96	110	94	100	108
21 %	127	85	87	60	66	70	70	75	81	102	93	114	108	110	110
22 %	124	98	77	50	77	75	83	77	79	94	90	107	94	94	90
23 %	109	94	96	62	87	72	87	94	89	87	89	92	85	75	67
24 %	131	81	87	77	83	75	79	85	87	103	87	111	93	93	95
25 %	104	97	98	65	67	64	74	91	65	72	91	74	81	72	64
26 %	124	91	84	80	84	108	86	80	93	91	104	91	64	73	82
27 %	98	107	94	57	59	55	57	103	64	68	87	74	75	72	68
28 %	109	96	94	92	83	94	100	87	94	81	96	85	96	96	94
29 %	122	82	96	80	82	86	90	84	88	86	86	88	88	84	82
30 %	106	98	94	71	84	84	94	98	90	88	88	96	88	90	94
31 %	108	92	98	49	71	84	82	88	86	82	90	84	65	73	78
32 %	112	105	81	46	60	75	87	81	81	85	77	79	72	74	74

APPENDIX I (continued)

G.P. #12 (Room Air)

FREQ	TS1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 %	103	84	111	72	100	92	84	80	107	82	76	84	66	43	60
2 %	108	87	103	110	87	130	135	110	119	119	101	67	78	67	56
3 %	91	91	116	105	132	97	102	86	127	110	110	118	89	100	56
4 %	131	91	77	88	100	117	115	117	117	77	66	62	91	71	71
5 %	114	88	97	92	88	128	111	145	121	97	78	92	92	61	80
6 %	82	87	130	70	73	109	116	107	116	100	107	91	103	59	57
7 %	124	114	60	80	92	86	76	98	100	82	74	78	64	74	90
8 %	85	118	95	72	97	85	89	102	108	110	89	93	79	83	83
9 %	88	113	97	62	93	81	93	111	95	95	97	104	79	95	86
10 %	100	96	102	46	73	90	94	92	94	102	96	90	73	79	73
11 %	78	116	104	40	73	92	97	107	111	109	107	107	92	90	73
12 %	100	78	120	0	48	65	83	89	98	94	94	91	87	70	67
13 %	91	78	130	0	18	51	62	57	74	74	68	78	72	57	55
14 %	112	95	91	0	16	43	58	58	68	64	66	70	70	72	66
15 %	96	89	113	0	24	32	45	43	63	65	59	74	52	59	56
16 %	110	108	80	0	10	36	44	56	54	58	56	70	54	54	58
17 %	104	114	80	0	25	38	53	68	69	68	64	75	53	58	58
18 %	103	75	121	0	22	46	60	60	70	77	66	77	66	46	52
19 %	105	97	97	0	25	43	62	60	69	60	64	71	64	64	69
20 %	112	97	89	0	29	38	59	65	76	74	72	78	68	63	55
21 %	94	119	85	20	47	63	78	87	78	81	81	81	72	63	63
22 %	88	123	88	21	47	69	88	88	92	90	92	86	79	77	69
23 %	98	92	109	18	50	67	81	86	90	86	81	86	60	71	52
24 %	106	114	78	25	42	74	78	91	87	89	76	74	59	78	76
25 %	103	99	97	17	43	64	77	73	90	97	86	90	56	58	53
26 %	104	113	82	15	39	50	76	76	67	89	84	84	69	73	71
27 %	106	104	88	12	24	52	62	68	74	78	80	92	80	70	70
28 %	102	105	91	21	37	54	70	78	83	91	94	98	96	78	67
29 %	98	102	98	14	25	44	65	67	73	71	83	90	79	73	69
30 %	93	100	106	37	48	63	81	96	87	85	83	85	85	80	91
31 %	105	93	100	34	45	77	96	105	107	96	91	89	91	87	91
32 %	96	98	105	19	36	62	81	81	91	100	103	98	88	84	86

APPENDIX I(continued)

G.P. #13(Room Air)

FREQ	TS1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 %	108	83	108	119	86	90	77	134	125	114	103	105	88	83	72
2 %	86	104	109	114	119	109	109	128	123	126	131	99	119	64	74
3 %	119	107	72	117	82	96	117	119	124	112	133	98	93	100	86
4 %	61	104	133	77	110	106	87	110	118	104	87	104	125	50	60
5 %	60	88	151	86	139	117	117	122	136	110	91	112	112	55	62
6 %	99	91	108	84	86	106	112	105	105	106	116	84	106	69	76
7 %	89	111	99	69	91	109	113	93	107	99	93	147	83	99	57
8 %	116	88	94	58	80	84	112	82	94	96	108	118	80	90	72
9 %	107	105	87	36	56	84	98	73	71	78	87	91	68	84	59
10 %	116	88	94	24	44	82	72	70	80	92	94	96	76	86	72
11 %	107	109	83	0	22	45	62	64	73	60	69	71	58	66	50
12 %	109	86	103	0	0	0	9	17	23	23	28	32	38	36	30
13 %	109	105	85	0	0	7	9	12	16	20	25	24	18	22	18
14 %	89	99	111	0	0	6	12	16	14	14	30	26	30	26	20
15 %	87	93	119	0	0	7	9	15	17	17	23	27	33	25	25
16 %	81	111	107	0	0	17	23	29	35	37	33	37	44	40	38
17 %	90	106	102	0	14	31	35	44	37	35	41	50	56	46	33
18 %	133	73	92	0	23	55	60	62	66	80	78	57	69	62	69
19 %	96	103	100	0	23	48	61	48	51	61	67	65	65	59	51
20 %	97	92	110	0	34	54	54	57	74	72	74	65	68	63	54
21 %	100	104	94	11	45	64	74	81	74	79	79	83	62	56	68
22 %	90	103	106	18	45	73	76	80	84	84	90	80	78	73	69
23 %	106	85	108	32	58	79	87	89	90	90	90	89	92	77	69
24 %	91	100	107	29	58	71	78	75	73	85	82	91	73	58	53
25 %	101	96	101	32	63	82	90	100	94	92	90	92	86	80	61
26 %	94	113	92	39	66	83	90	100	103	101	86	105	79	71	67
27 %	100	92	106	39	61	82	86	94	92	94	110	106	86	82	73
28 %	96	100	102	35	62	76	89	98	96	91	96	102	100	91	76
29 %	100	98	102	36	48	66	80	82	80	78	88	92	88	86	82
30 %	99	99	101	45	42	42	42	101	101	99	110	113	107	113	96
31 %	98	113	87	62	73	80	80	73	102	120	120	120	109	106	102
32 %	100	104	95	63	72	72	77	95	95	122	122	131	131	122	90

APPENDIX J

ANALYSIS OF VARIANCE ON N_1 AMPLITUDE

(2-way, Repeated Measures on B, Test Sequence)

A. Frequencies 1-32 (2.1-30 kHz).

	SS	DF	MS	F	p
BET SUBJ	14218.882	9		.81	ns
A	1309.441	1	1309.441		
ERROR	12909.440	8	1613.680		
WITH SUBJ	16721.936	110			
B	10841.390	11	985.580	18.85	<.001
AB	1281.398	11	116.490	2.22	<.05
ERROR	4599.147	88	52.263		

B. Frequencies 10-22 (4.5-12.8 kHz).

	SS	DF	MS	F	p
BET SUBJ	13871.821	9			
A	5320.008	1	5320.008	4.97	ns
ERROR	8551.813	8	1068.976		
WITH SUBJ	32820.150	110			
B	25805.039	11	2345.912	32.96	<.001
AB	752.951	11	68.450	.96	ns
ERROR	6262.158	88	71.160		

C. Frequencies 13-18 (5.9-9.1 kHz).

	SS	DF	MS	F	p
BET SUBJ	14645.536	9	7498.683	8.39	<.05
A	7498.683	1	893.356		
ERROR	7146.853	8			
WITH SUBJ	38552.536	110			
B	31486.596	11	2862.417	40.66	<.001
AB	871.969	11	79.269	1.12	ns
ERROR	6194.246	88	70.389		

D. Frequencies 14-16 (6.4-7.6 kHz).

	SS	DF	MS	F.	p
BET SUBJ	17984.397	9			
A	9023.736	1	9023.736	8.05	<.05
ERROR	8960.660	8	1120.082		
WITH SUBJ	41375.321	110			
B	33967.914	11	3087.992	42.28	<.001
AB	980.619	11	89.147	1.22	ns

APPENDIX J (cont.)

E. Frequencies 1-12 (2.1-5.4 kHz)

	SS	DF	MS	F	p
BET SUBJ	22956.089	9			
A	728.161	1	728.161	.26	ns
ERROR	22227.928	8	2778.491		
WITH SUBJ	14483.450	110			
B	6049.949	11	549.995	6.61	<.001
AB	1119.916	11	1001.810	1.12	ns
ERROR	7313.583	88	83.108		

F. Frequencies 19-32 (9.9-30 kHz).

	SS	DF	MS	F	p
BET SUBJ	18168.353	9			
A	507.174	1	507.174	.22	ns
ERROR	17661.179	8	2207.647		
WITH SUBJ	21278.904	110			
B	12762.894	11	1160.263	16.65	<.001
AB	2347.220	11	213.383	3.04	
ERROR	6168.788	88	70.099		

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