COMBINED EFFECTS OF NOISE AND NEOMYCIN: COCHLEAR CHANGES IN THE GUINEA PIG

by

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INTRODUCTION

The purpose of this work was to investigate a possible ototoxic interaction between neomycin and excessive acoustic stimulation, both of which are known to damage the cochlea. Cochlear damage in guinea pigs was measured electrophysiologically and histologically. For the purpose of this work, an interaction between the two agents when given in combination was defined as an effect exceeding the additive sum of the effects of neomycin or noise when given alone.

I. An Overview of Some Ototoxic Agents:

Transient as well as permanent hearing loss is known to result from exposure to a variety of agents. The majority of these are pharmacological compounds such as the salicylates, anti-thyroid drugs, quinine, loop-inhibiting diuretics and the aminoglycoside antibiotics. Hearing loss may also result from acoustic overexposure, a common environmental agent. Along with transient or permanent hearing loss, in humans the clinical syndrome of tinnitus may be produced.

Our lives are filled with usually inconsequential exposure to many of these agents. The use of aspirin in the relief of minor pain is extremely widespread. Except in high doses, tinnitus or other otological problems rarely occur. Less common in use than aspirin, but still used widely in the hospital setting, are the aminoglycoside antibiotics.

The aminoglycoside antibiotics are often essential for the treatment of life threatening infections. According to estimates, within hospitals alone, one million patients each year are treated with one

of this class of compounds.* The reported incidence of ototoxicity ranges from 0.5% to 55% of the patients treated. Additionally, these antibiotics are often contained in many non-prescription preparations for topical application.

Outside of the hospital, the technological advances of modern society have provided sources of noise pollution that are louder than those for which nature seems to have prepared the ear. Naturally occurring sounds rarely approach levels of more than 100 dB SPL,** yet in our factories, airports, wars, city streets and highly amplified concerts acoustic sound levels far greater than 100 dB are reached. Man has surpassed nature in the intensity of sounds that he is able to produce but has not been overly successful in controlling their level or prevalence.

The legislative limits set for noise by the Occupational Safety and Health Act of 1970 fix the upper levels of permissible exposure for industrial workers at 90 dB (A)*** for 8 hours, 95 dB (A) for 4 hours, 100 dB (A) for 2 hours, and up to 115 dB (A) for 15 minutes. In theory, at least 15% of the individuals exposed at these levels will develop some hearing loss (Falk, 1972). These figures ignore the possibility of concurrent exposure to other ototoxic agents.

The justification for the study of potential interactions between the aminoglycoside antibiotics and noise is provided by the prevalence of both of these agents in medical therapeutics and modern life, and

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*R.E. Brummett, Personal Communication, 1978

**dB SPL (Sound Pressure Level) is referred to 0.0002 dynes/cm^2

***dB (A) indicates that the sound level was obtained using a filter which discriminates against the low frequencies.

by their common effects on the cochlea. Concurrent exposure to the antibiotics, in topical or systemic application, and to noise, such as urban traffic, hospital respirators, incubators and high speed surgical drills, may occur frequently. It is therefore of extreme interest and relevance to examine the possible interaction between the aminoglycoside antibiotics and excessive acoustic exposure.

II. The Aminoglycoside Antibiotics:

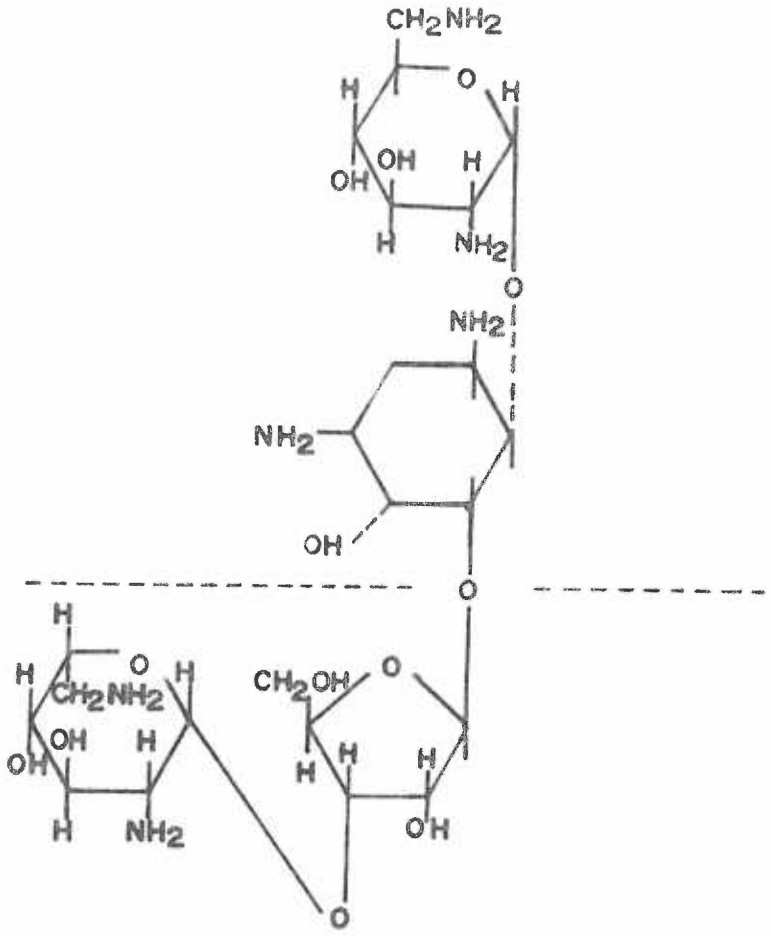
The aminoglycoside class of antibiotics was first introduced with streptomycin by Schatz, Bugie and Waksman (1944). Since that time research has generated many more such compounds. In approximate order of their discovery these include neomycin, kanamycin, gentamicin, tobramycin and amakacin. As suggested by the name aminoglycoside, the general structure of these drugs consists of amino sugars in glycosidic linkage. The structure of Neomycin B may be seen in Figure 1. The aminoglycoside antibiotics are highly polar (cationic) and this quality is responsible for many of their pharmacodynamic properties. These include: poor lipid solubility, poor absorbtion from the gastrointestinal tract, poor penetration of the cerebrospinal fluid and rapid elimination by the normal kidney (Goodman & Gilman, 1975).

The aminoglycoside antibiotics have proven to be invaluable in the treatment of tuberculosis and gram-negative bacterial infections. These drugs inhibit bacterial protein synthesis by attachment to the 30S bacterial ribosomal subunit (Goodman & Gilman, 1975). Except in the mitochondria, ribosomes of mammalian cells are composed of different subunits. Although the aminoglycoside antibiotics have some effects on mammalian protein synthesis, the differences between

FIGURE 1.

General structure of the Neomycin B molecule.

Note high polarity, amino sugars in glycosidic linkage.


CH2NH2
O
H
H
OH
OH
H
H
NH2
O
NH2
NH2
OH
O
H
H
O
CH2NH2
OH
H
OH
H
NH2
CH2OH
O
H
H
H
OH
O

mammalian and bacterial ribosomes imply another mode of action (Beard, Armentrout & Weisberger, 1969). Some of the aminoglycoside antibiotics, including neomycin, kanamycin and streptomycin, may produce neuromuscular and ganglionic blockade (Pittinger & Adamson, 1972). When given systemically this may lead to a "curariform" respiratory arrest. However, it has been stated that the only tissues adversely affected after systemic administration of the aminoglycoside antibiotics are the inner ear and kidney (Hawkins, 1970). Some evidence points to action of these drugs on the cell membranes (Schacht, 1976). The aminoglycoside antibiotics are drugs of choice for pseudomonas aeruginosa infections, bubonic plague, bacterial endocarditis and meningitis due to susceptible organisms.

The usual route of administration of the aminoglycoside antibiotics is an intramuscular injection. Once in the bloodstream elimination of the drug is dependent on the kidney. Following oral administration, usually given in preparation of the bowel for surgery or in cases of advanced liver disease, the drugs are poorly absorbed and almost completely eliminated (97%) in the feces. The antibiotics are also used topically as ointments and soaks for wound irrigation and burns. The drugs are sometimes mistakenly presumed not to be absorbed in appreciable amounts from the latter two sites of administration.

The nephrotoxic quality of the aminoglycoside antibiotics place further problems in their use. In the kidney the drugs are filtered into the glomerulus and not reabsorbed. The clearance of the drugs approaches the glomerular filtration rate (GFR). A renal tubular necrosis may be induced by the drugs leading to reduced GFR and the accumulation of excessively large blood concentrations. In the

treatment of individuals in renal failure, high levels of the drugs may accumulate due to an already reduced GFR (Harris, 1972; Brummett, 1973). Reports indicate that enough of the drugs may be absorbed from topical or oral administration to lead to toxic levels in patients in renal failure (Fields, 1964; Gibson, 1967; Dayal, Smith, & McCain, 1974). Ototoxicity resulting from topical administration of eardrops containing neomycin to the intact middle ear of guinea pigs has also been reported (Brummett, Harris & Lindgren, 1976).

III. Ototoxic Effects of the Aminoglycoside Antibiotics:

The toxic effects on the ears of animals exposed to the aminoglycoside antibiotics include morphological damage to the sensory hair cell populations of the organ of Corti as well as changes in electrophysiological measures of cochlear function. Although these effects are consistent within the general class of compounds, different drugs vary in regard to relative ototoxicity. In approximate order from the most to the least toxic at the recommended clinical dosages are: neomycin, kanamycin, streptomycin and gentamicin (Leach, 1962; Harris, 1972; Brummett, 1973).

In general, histopathological changes occur first in the innermost row of the three rows of outer hair cells (OHC's) in the basal turn of the cochlea (Reddy & Igaroshi, 1962). A concurrent early change is a disturbance of the orderly "W" pattern of the stereocilia of the OHC's in this area (Hawkins & Engstrom, 1964). With continued exposure, disarrangement of the double membrane along the sides of the OHC's becomes evident and dark inclusion bodies appear in subcuticular cytoplasm (Lim & Melnick, 1971). Cells become distended and finally

the plasma membrane ruptures and the cells disintegrate completely (Ylikoski, 1974). Losses of OHC's in the inner rows at the base are followed by destruction of OHC's in the outer rows (toward the stria vascularis) and by cells further toward the apex (Ward & Fernandez, 1961; Benitez, Schuknecht & Brandenburg, 1962). With continued exposure the single row of inner hair cells (IHC's) may be lost (Waitzova, Kyndand & Sobek, 1964).

Histopathology correlates well with depression of the AC cochlear potential according to the degree of damage and the location of that damage within the cochlea. As the basal turns of the cochlea are responsible for the transduction of high frequency sound (Helmholtz, 1930; Bekesy, 1960), damage to the basal turn is reflected in depression of cochlear potentials in the high frequencies (Stebbins, Miller, Johnsson & Hawkins, 1969). With continued drug exposure and damage further toward the apex of the cochlea, lower frequencies become affected (Gibson, 1967; Brummett, 1973). A reliable correlation between hair cell loss and degree of hearing loss has been reported (Ylikoski, 1974).

IV. Otological Effects of Acoustic Overexposure:

The damaging effects of noise exposure on the ear have been recognized since 1831 when Fosbroke (Fosbroke, 1831) mentioned a hearing loss among blacksmiths and artillerymen in the first volume of the Lancet. Already at this time he had differentiated between the hearing loss following exposure to the noise of guns from that following a lifetime spent working in the smithy. In 1890 Habermann (Habermann, 1890) published histological findings on the temporal

bones of an old man who, unable to work due to noise induced hearing loss, was hit by a train that he failed to hear. Habermann noted defects of the organ of Corti in the basal turn of the cochlea.

Damage to the ear resulting from noise exposure depends on several variables: the nature of the sound as well as the intensity and duration of exposure. Types of sound may be classified as pure tone, impulse, broad band or narrow band noise. In general, environmental noise consists of a mixture of the above.

Exposure to all types of sound will lead to destruction of hair cells of the organ of Corti if presented at sufficient intensity. Damage due to noise exposure, as with the aminoglycoside antibiotics, begins in the outer hair cells, then proceeds to the inner hair cells. Some evidence exists that the area of greatest loss of OHC's within the cochlea corresponds roughly to the frequency spectrum of the sound used for acoustic exposure (Stockwell, Ades & Engstrom, 1969; Engstrom, Ades & Bredberg, 1970). However, this is not in exact correspondence with the place theory of frequency discrimination and Greenwood's mapping of the cochlea (Culler, 1935; Galambos & Davis, 1943; Fernandez, 1952; Greenwood, 1961). Broad band noise, which includes a large range of frequencies, instead of evenly affecting OHC's throughout the cochlea, preferentially affects the upper basal and lower 2nd turns in the guinea pig (Spoendlin, 1971). This area is thought to correspond to maximum basilar membrane displacement at 4 kHz and seems to be an especially sensitive portion of the guinea pig cochlea (Spoendlin, 1976).

The maximum depression of the human audiogram due to pure tone exposure lies, in general, one-half to one octave above the exposure

frequency (Kellerhals, 1972). Moreover, the depression of the audiogram tends to approach the 4 kHz range from frequencies of exposure above and below this. This may lead to the so-called "4 kilohertz notch" in the audiograms of noise exposed humans. Electrophysiological measures of damage due to broad band noise reveal primary depression of the AC cochlear potential in the 4 kHz areas as well (Kellerhals, 1972; Fried, Dudek & Bohne, 1976). With longer durations of exposure to broad band noise, the isopotential functions of the AC cochlear potential are depressed over a larger frequency range (Lawrence & Yantis, 1957).

In charting the course of damage within the cochlea due to noise exposure, Engstrom, Ades & Bredberg (1970) note OHC swelling, nuclear pyknosis and disintegration with myelin figures and vacuoles forming in cell cytoplasm as a primary change. Spoendlin (1971) notes swelling of the OHC's, swelling of the dendrites to both OHC's and IHC's with eventual cell rupture and retrograde nerve degeneration. Degeneration of nerve fibers following noise exposure has also been noted by Wright (1976) even in the absence of OHC loss. Lim & Melnick (1971), using electron microscopy, have noted an increase in 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 cuticular plate deformation leading to eventual cell rupture.

Following the initial trauma to the ear, there may be some recovery of function over time and it has been suggested that the eventual permanent loss of function of an ear is determined by its ability to recover rather than by the initial loss (Lawrence & Yantis,

1957).

Recent reviews have ascribed damage due to loud noise exposure to two mechanisms, depending on the intensity of the sound (Hammernik & Henderson, 1976). At intensities above 125 dB, direct mechanical destruction and metabolic cellular exhaustion may be competing factors in hair cell loss. At lower intensities of sound metabolic effects are predominant and structural alterations appear only as secondary manifestation of the metabolic disturbance. In general, after a 30 second exposure above 125 dB, the organ of Corti will be entirely thrown off the basilar membrane at the area of peak mechanical displacement of the basilar membrane. This indicates a mechanical effect with the primary site of damage corresponding to the frequency of stimulation (Fried, Dudek & Bohne, 1976). With longer exposures of lesser intensity, rearrangement of cellular contents, swelling of hair cells and afferent dendrites with eventual hair cell rupture suggest a metabolic source of damage (Spoendlin, 1976; Hammernik & Henderson, 1976; Stockwell, Ades & Engstrom, 1969).

V. Otological Interactions Between the Aminoglycoside Antibiotics and Noise:

An interaction between different agents, or between an agent and physiological state of an organism, may lead to augmentation or diminuation of the response. Interactions between different drugs, between drugs and physiological state, and between drugs and noise may affect the ear. For example, if an aminoglycoside antibiotic is administered to a patient with poor renal function, an augmentation of cochlear damage may be noted due to the interaction of the drug with the state of the kidney (reduced glomerular filtration). In a

similar sense, a nephrotoxic and ototoxic drug may lead to an augmentation of cochlear damage through a vicious cycle of nephrotoxicity and ototoxicity. In this report the concern is with the interactions when two different agents, both having an effect on the same organ, are given simultaneously.

An ototoxic interaction leading to augmentation of damage effects has been reported between kanamycin and ethacrynic acid, a potent diuretic (West, Brummett & Himes, 1973; Brummett & Brown, 1975). It was found that in dosage levels which alone caused no damage, the combination led to severe and permanent cochlear damage. Several investigators have failed to demonstrate an interaction between the salicylates and noise (Mitchell, 1973) or between the loop-inhibiting diuretics and noise (Vernon, Brown & Brummett, 1976). Interactions have been demonstrated between some of the aminoglycoside antibiotics and noise (Gannon & Tso, 1969; Dayal, Kokshanian & Mitchell, 1971; Jauhiainen, Kohonen & Jauhiainen, 1972; Dayal & Barek, 1975; Hawkins, Marques & Clark, 1975; Marques, Clark & Hawkins, 1975).

Kanamycin has received the largest amount of attention regarding possible interactions with noise. Gannon and Tso (1969) found that noise exposure resulting from 42 shots of a 0.32 calibre pistol in combination with kanamycin led to a greater loss of OHC's in guinea pigs than that resulting from either agent when given alone.

Dayal, Kokshanian and Mitchell (1971) report an interaction between relatively low intensity noise (68 dB - 72 dB) and low doses of kanamycin (15 - 50 mg/kg) in guinea pigs using OHC counts as a measure of damage. The low intensity noise was presented continuously for 3 - 5 weeks and was produced by an infant incubator. Kanamycin

was given in daily injections during this time. An interaction was identified in that the animals exposed to a combination of agents showed OHC losses whereas acting alone, neither of the two agents produced any OHC losses.

An augmentation of damage was reported by Dayal and Barek (1975) in guinea pigs exposed to 5 weeks of white noise of moderate intensity (90 dB) in combination with daily injections of kanamycin (100 mg/kg). In this study an interaction was identified by a significant difference in OHC losses between animals exposed to the combination and those exposed to the single agents.

More recent work has not completely confirmed the findings described in these studies. Hawkins, Marques and Clark (1975), using both electrophysiological and histological measures, report extremely variable findings after treating guinea pigs with various combinations of kanamycin (100 mg/kg) and noise (octave and broad band at 90 dB, 100 dB and 106 dB) for seven days. Their findings indicated that interactions between noise and kanamycin were seen only in some animals and only with noise exposures of at least 100 dB.

Marques, Clark and Hawkins (1975) in a similar study have identified an interaction using octave band noise and high doses of kanamycin (200 mg/kg). It was found that the pathological changes were the greatest when both agents were given concurrently, or within an interval of one week. It appears that both duration of exposure and intensity are important variables in any possible interactions.

Unpublished observations from the Kresge Hearing Research Laboratory* using combinations of pure tone acoustic exposure with

*Vernon, J.A., Meikle, M.B., Brummett, R.E. Personal Communication, 1976

kanamycin have failed to demonstrate any interaction between these agents. In these experiments the animals were anesthetized and, although very intense (115 dB - 130 dB), the pure tone exposure durations were brief ($\leq$ 30 min.).

Neomycin is the only other aminoglycoside antibiotic that has been studied in combination with noise. In that study (Jauhiainen, Kohonen & Jauhiainen, 1972) an augmentation of damage was reported using electrophysiological and histological measures in guinea pigs. Animals were exposed to an octave band of noise centered at 8 kHz of high intensity (115 dB) for a total of 70 hours over a 7 day period in combination with daily injections of neomycin sulfate (200 mg/kg). The relatively high frequency of acoustic exposure in this study was chosen to maximize damage in the basal turn of the cochlea, the area most sensitive to the aminoglycoside antibiotics.

In the group that received the combination of agents, the reduction of the AC cochlear potential at 250 Hz, 500 Hz, 1 kHz, 2 kHz and 4 kHz was found to be greater than that for either agent when acting alone. Counts of OHC's in these animals indicated the same effect. An interaction was identified by the authors in that the combination of noise and neomycin produced greater OHC damage than would have been predicted on the basis of simple addition of the effects of either agent given alone.

There are several factors in the study by Jauhiainen et al which render their results open to question:

1). Only two weeks were allowed for stabilization of damage effects following exposure. Although the majority of effects should have been resolved by this time, it is known that continued damage

and substantial recovery may continue to occur for up to 30 days following exposure to noise (Lawrence & Yantis, 1957; Bohne, 1974) or to ototoxic antibiotics (Hawkins et al, 1964).

2). The methodology used for the recording of the AC cochlear potential was not adequately reported. Additionally, no measures were made at frequencies above 4 kHz. This seems an oversight considering the prevalence of high frequency hearing loss due to neomycin and the fact that an octave band of noise centered at 8 kHz was used for the acoustic exposure. No measures were made of standing wave patterns in the exposure cage.*

3). No attempt was made in the study to control for procedural variables. The group that received the noise alone and the control group did not receive sham injections (e.g. saline), nor was any attempt made to specify the ambient noise experienced by the control group or the group that received neomycin alone.* Most importantly, a blind procedure was not employed, either for injections or measurements.

Due to the conflicting evidence concerning the possible interactions between aminoglycoside antibiotics and acoustic exposure, it was decided to investigate possible interactions in this laboratory, using neomycin and noise at levels similar to those employed by Jauhiainen et al (1972) in a well controlled study with extended measures of cochlear function.

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*Jauhiainen, T., Personal Communication, 12-29-77.

MATERIAL AND METHODS

I. Animals:

Thirty-two healthy pigmented guinea pigs were selected from the stock maintained at the Kresge Hearing Research Laboratory, University of Oregon Health Sciences Center. This "Topeka strain" has an excellent history of freedom from middle ear disease (Walloch, Brummett & Himes, 1976). All animals weighed between 200 - 400 grams and were shown to have auditory function before the procedure by exhibiting the Preyer pinna reflex. The animals were randomly assigned into two groups to receive either high or low intensity sound exposures. These groups were then subdivided to receive either neomycin sulfate or saline (vehicle) injections by an associate so as to maintain a blind procedure with respect to the drug. The four groups thus assigned were as follows:

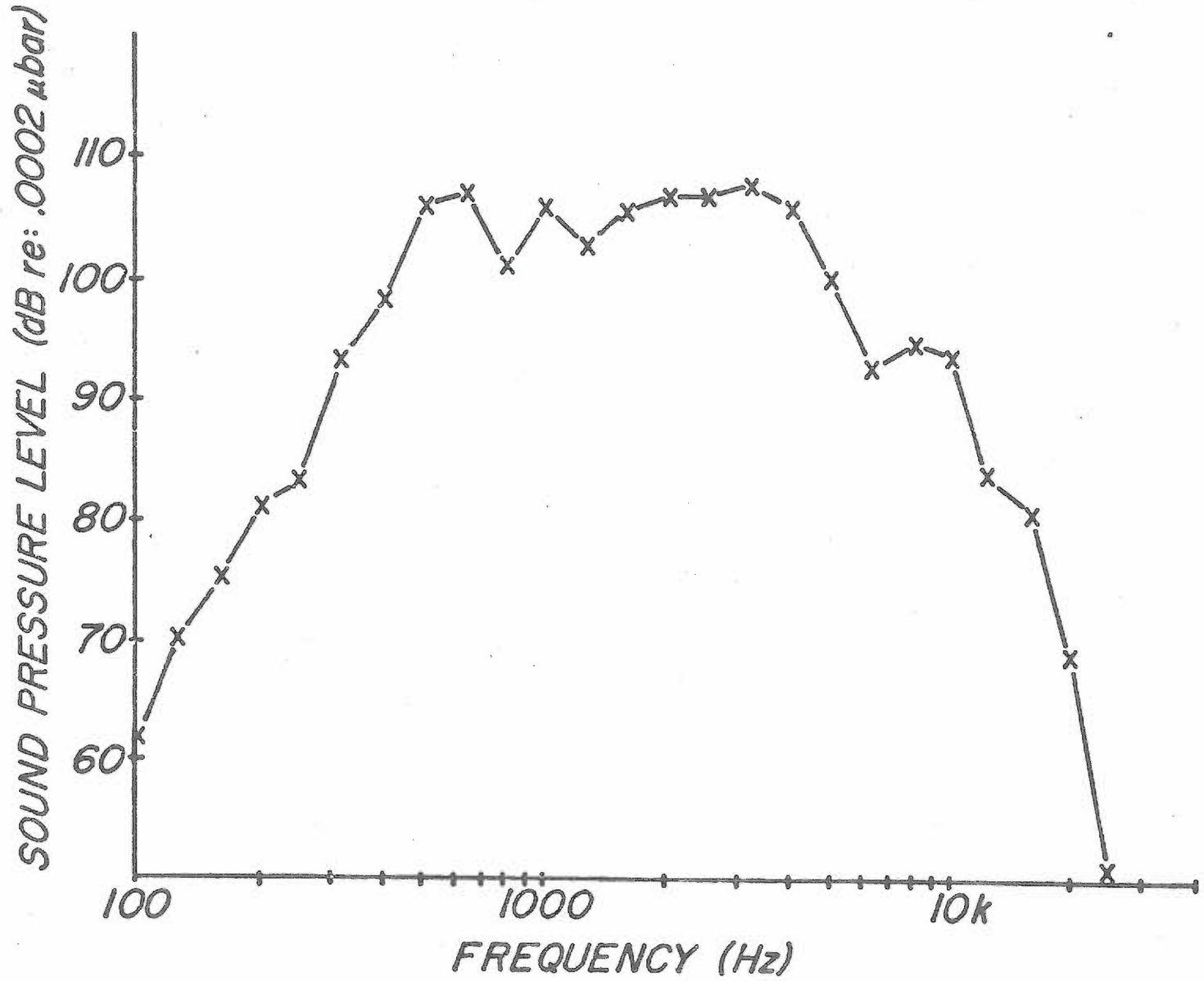
Group	Number of animals	Acoustic Exposure	Drug Treatment
Combination	8	115 dB*	neomycin sulfate (200 mg/kg)
Noise-alone	8	115 dB	normal saline
Neomycin-alone	8	45 dB	neomycin sulfate (200 mg/kg)
Control	8	45 dB	normal saline

A full table of the weight of each animal over the course of the procedure with the group assignments is available in Appendix A.

*Sound Pressure Level is referred to 0.0002 dynes/cm^2

FIGURE 2.

One-third octave bandwidth frequency power spectrum of the broad band of white noise used as acoustic exposure. Measurements were made at the center of the exposure cage at 115 dB SPL. Data points are at the center frequency of each one-third octave.


SOUND PRESSURE LEVEL (dB re: .0002 μbar)
110
100
90
80
70
60
100
1000
10k
FREQUENCY (Hz)

II. Acoustic Exposure:

The acoustic exposure was a broad band of noise. The frequency power spectrum of this noise, measured in 1/3 octave bandwidths is shown in Figure 2. The spectrum was measured at the center of the exposure cage at approximately animal head height (3 cm). The spectrum was relatively even in sound pressure from 300 Hz through 4 kHz, dropping substantially in energy beyond these points.

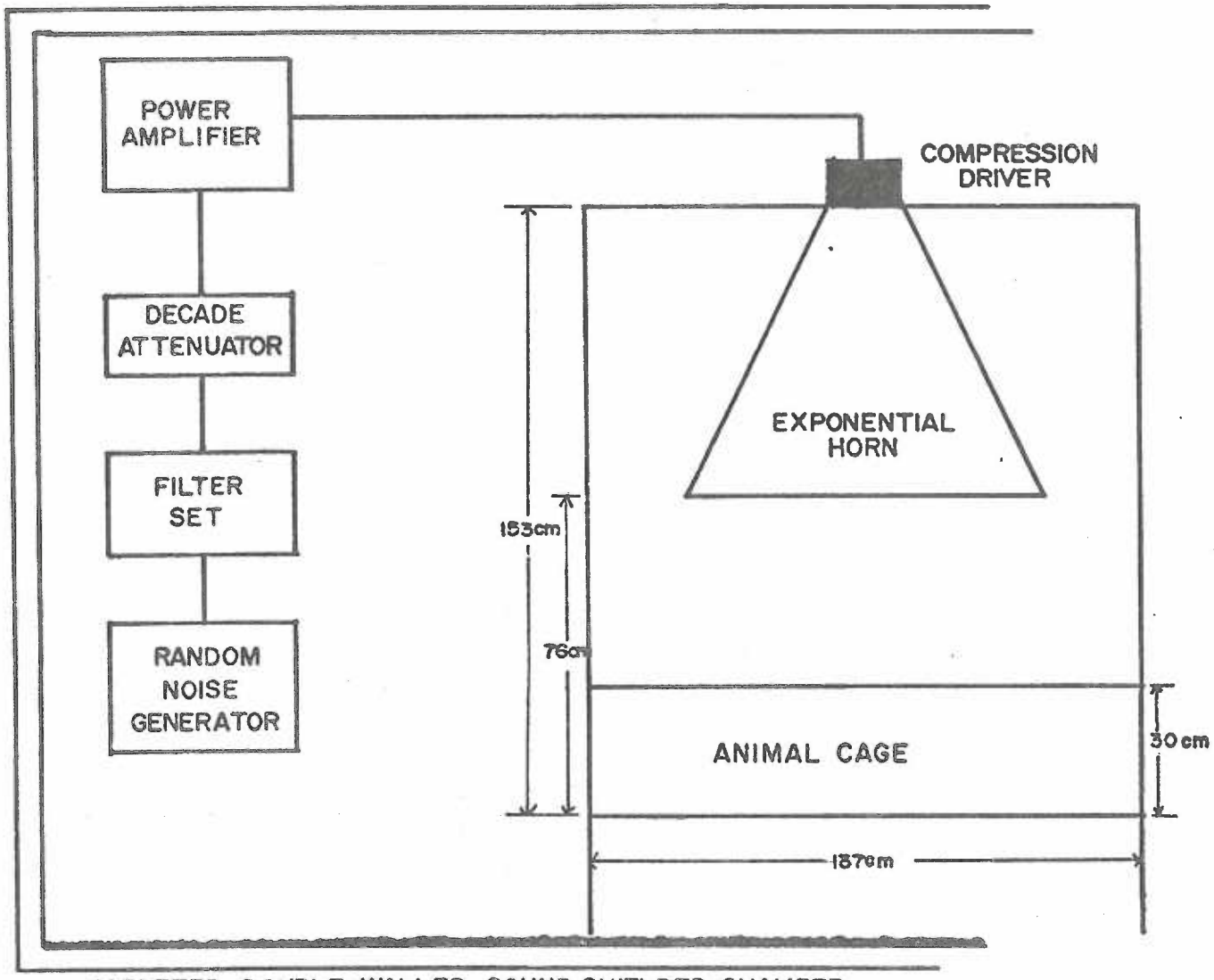
The 115 dB exposure was chosen to be consistent with earlier work (Jauhiainen et al, 1972), although a broad band of noise rather than an octave band was used in this study. The low level exposure was well below the ambient level in the guinea pig colony and was added to provide an innocuous sound environment as a procedure control.

Exposure to noise was maintained for 10 hours each day over seven consecutive days. During sound exposure the animals were confined as a group of 16 within a wire mesh cage described below. Noise was produced by a single J. B. Lansing compression driver (2482). This was driven by a random noise generator (General Radio 1382) in conjunction with a filter (Bruel & Kjaer 2112), a decade attenuator, and a Dynaco 60 watt power amplifier. The compression driver was suspended 153 cm above the cage floor and was fitted with an exponential horn whose open end terminated 76 cm above the cage floor (Figure 3).

The sound exposure cage was designed to provide at least 60 in^2 per animal (Institute of Laboratory Resources, 1968), and as uniform a sound field as possible throughout. It was constructed of lightweight wood and 0.64 cm wire mesh. The dimensions were 137 cm (length) by 46 cm (width) by 30 cm (height). The speaker was suspended at an adjustable height above the cage floor allowing for adjustment for a

FIGURE 3.

Schematic diagram of the sound exposure cage, sound producing apparatus and double-walled sound shielded chamber. Dimensions of the sound cage are indicated in centimeters.


POWER AMPLIFIER
DECADE ATTENUATOR
FILTER SET
RANDOM NOISE GENERATOR
COMPRESSION DRIVER
EXPONENTIAL HORN
153cm
76cm
ANIMAL CAGE
30cm
137cm
CARPETED, DOUBLE-WALLED, SOUND SHIELDED CHAMBER

homogenous sound field. The entire cage was suspended 30 cm above the carpeted floor of a double-walled sound shielded room (Industrial Acoustics Company). Water bottles were attached at both ends of the cage.

Sound pressure levels were measured with a sound level meter (Bruel & Kjaer 2203) on the "Linear" setting for the 115 dB sound; it was necessary to use the "C" scale for measuring the 45 dB sound to overcome signal to noise problems on the "Linear" scale due to ambient low frequency vibration. For calibration of the 115 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 (624 points). The sound pressure averaged over all these points was 114.95 dB and within a range of 113.4 dB to 116.5 dB. The low intensity sound was obtained by adjusting the decade attenuator for a 45 dB sound pressure level measured at the center of the cage.

III. Injections:

A solution of neomycin for injection was prepared by mixing 12.89 grams of neomycin sulfate powder* (Upjohn) with normal saline to obtain a neomycin solution of 200 mg/ml. The solution of neomycin was then stored in the refrigerator with a bottle of an equal volume of normal saline (45 ml). Dosages were easily calculated from the weight of the animal for both solutions.**

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* 0.698 grams of neomycin base per gram of neomycin sulfate. Neomycin molecules A, B, and C contained in this preparation in order of increasing amounts.

**The volume then being 1 ml/kg to achieve a dose of 200 mg/kg.

Animals were weighed on day 1, 4 and 7 (Appendix A). Drug dosages were recalculated accordingly at these times. To maintain blind procedure with respect to the drug, syringes were labeled with animal ear tag numbers and were loaded by associates for later injection each day.

IV. Procedure:

The drug and noise exposures were given concurrently to each group of animals over a period of seven days. Injections of neomycin or of normal saline (drug vehicle in equivalent volume) were given subcutaneously immediately before the animals were placed in the sound exposure cage each evening about 11:00 P.M. Following 10 hours of sound exposure, the animals were removed from the exposure cage and returned to their home cages. The entire procedure involved two weeks, the first for injections and noise exposures for the 16 animals receiving high intensity sound, the second week for injections and noise exposures for the 16 animals receiving low intensity sound. The following is a schematic of the experimental design:

	ACOUSTIC EXPOSURE	
	WEEK 1 - 115 dB	WEEK 2 - 45 dB
DRUG TREATMENT: Saline	Noise-alone Group (8 animals)	Control Group (8 animals)
DRUG TREATMENT: Neomycin	Combination Group (8 animals)	Neomycin-alone Group (8 animals)

Following each week of exposures the animals in that group were return to their home cages for 30-40 days before testing.

The experimental procedure was run in a blind fashion with respect to the drug but not to the sound. All measures, electrophysiological and histological, were also run blind with respect to this agent. Testing of the two groups of 16 animals followed the same temporal order as the treatments. The total number of injections was seven for each animal. The total time spent in the noise exposure cage was 70 hours for each animal.

Water was available at all times in both the sound exposure cage and in the animals' home cages. Food was only available in the home cages where the animals spent 14 hours of each exposure day. Following the seven day treatment period during the 30-40 day interval which was allowed for damage effects to stabilize, the animals were kept in their home cages with full access to food and water. No further exposure to drugs or to sound (other than the ambient sound level within the laboratory) was given during this time.

V. Preparation for Electrophysiological Testing:

At the end of the stabilization period, each of the animals was prepared for the recording of the AC cochlear potential at the round window. 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 immediately placed on artificial ventilation. The pinnae were excised bilaterally. A heating pad was used to maintain rectal temperature at 38° C $\pm$ 2°. The animals were then prepared for the recording of the AC cochlear potential using the postauricular approach (Vernon & Meikle, 1974) as follows:

With visualization through an operating microscope, the thin layer of muscle covering the bulla was cleared away by blunt dissection. With the tip of a pointed scalpel a small opening was made in the soft bone of the bulla; this was enlarged until the round window was exposed to view. 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 used. A specially designed sound cannula (Vernon & Meikle, 1974) was sealed into the external auditory meatus with stop cock grease. The cannula contained provision for a calibrated microphone (Bruel & Kjaer, 1/2 inch) in conjunction with a calibrated probe tube (1 mm). The sound cannula was connected to a loudspeaker (General Radio 555) by 25 cm of soft rubber tubing and 35 cm of rigid-walled tubing. The microphone was inserted into the cannula with the probe tube barely projecting into the external auditory meatus before the test procedure. It was left in during all subsequent electrophysiological procedures on that ear 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 a micromanipulator to contact the round window membrane. A second active electrode was placed 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 DC powered high gain differential amplifier (Princeton Applied Research) where they were amplified 1000 times and led to a narrow band wave analyzer (General Radio 1900-A). Figure 4 shows a schematic diagram of the entire preparation.

VI. 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, 3, 4, 5, 7, 8, 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. When corrected for the actual sound pressure in each ear, the 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 6 dB steps) until the maximum output of the ear was reached was obtained at 1 kHz and at 10 kHz. From these measures intensity functions could be constructed for each ear with a record of the maximum electrical output attainable for that ear. All electrophysiological measures were recorded in RMS voltages. Tests were periodically made for radiation artifact from the loudspeaker.*

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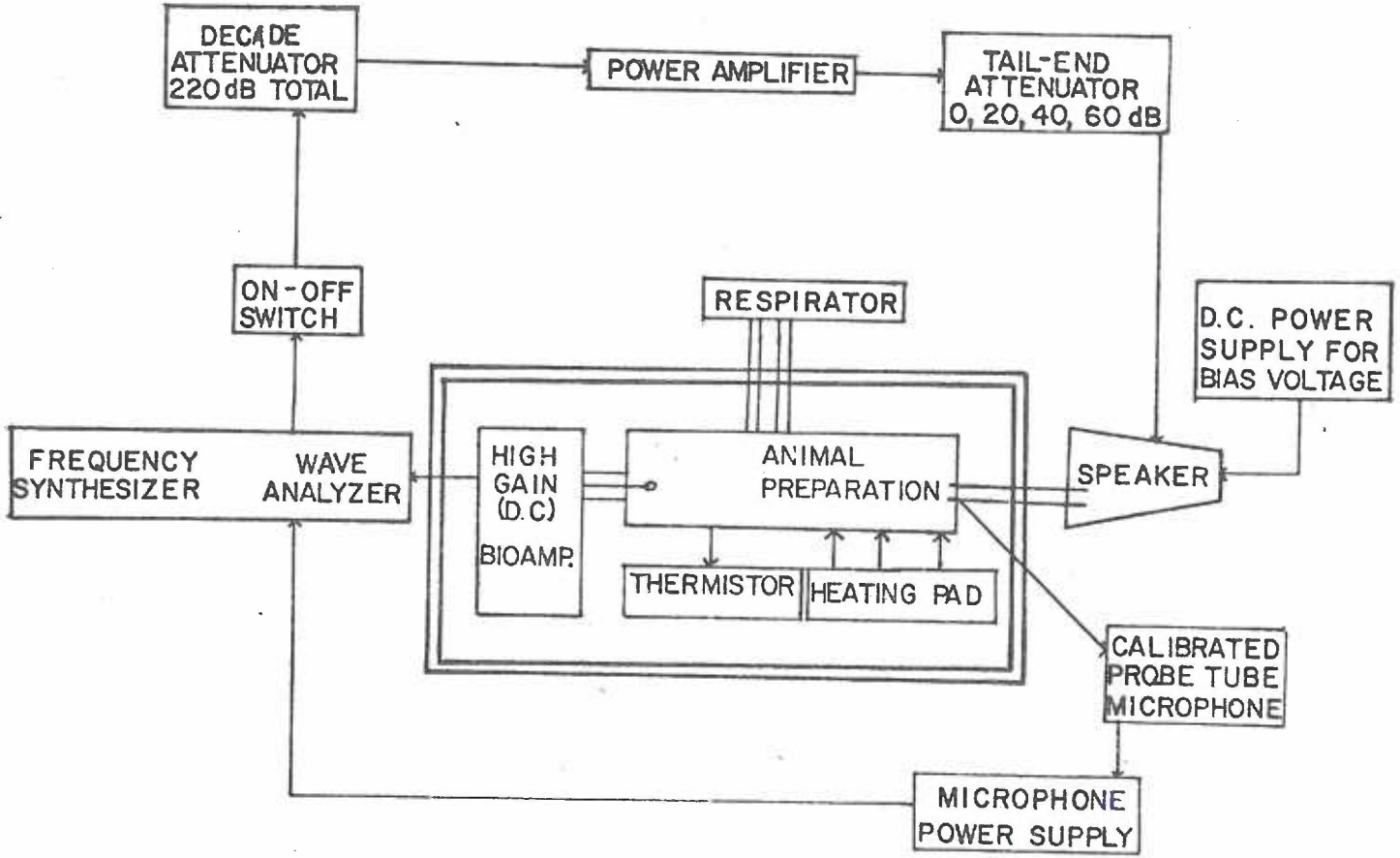
* Two methods were used: 1. Replacement of the round window electrode to adjacent healthy tissue, or 2. Removal of the DC bias on the loudspeaker thus decreasing the sound intensity while preserving the electrical radiations.

FIGURE 4.

Schematic diagram of the sound producing system, animal and biological recording system used for the electrophysiological measurements. 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 220 dB 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

VII. Histological Measures:

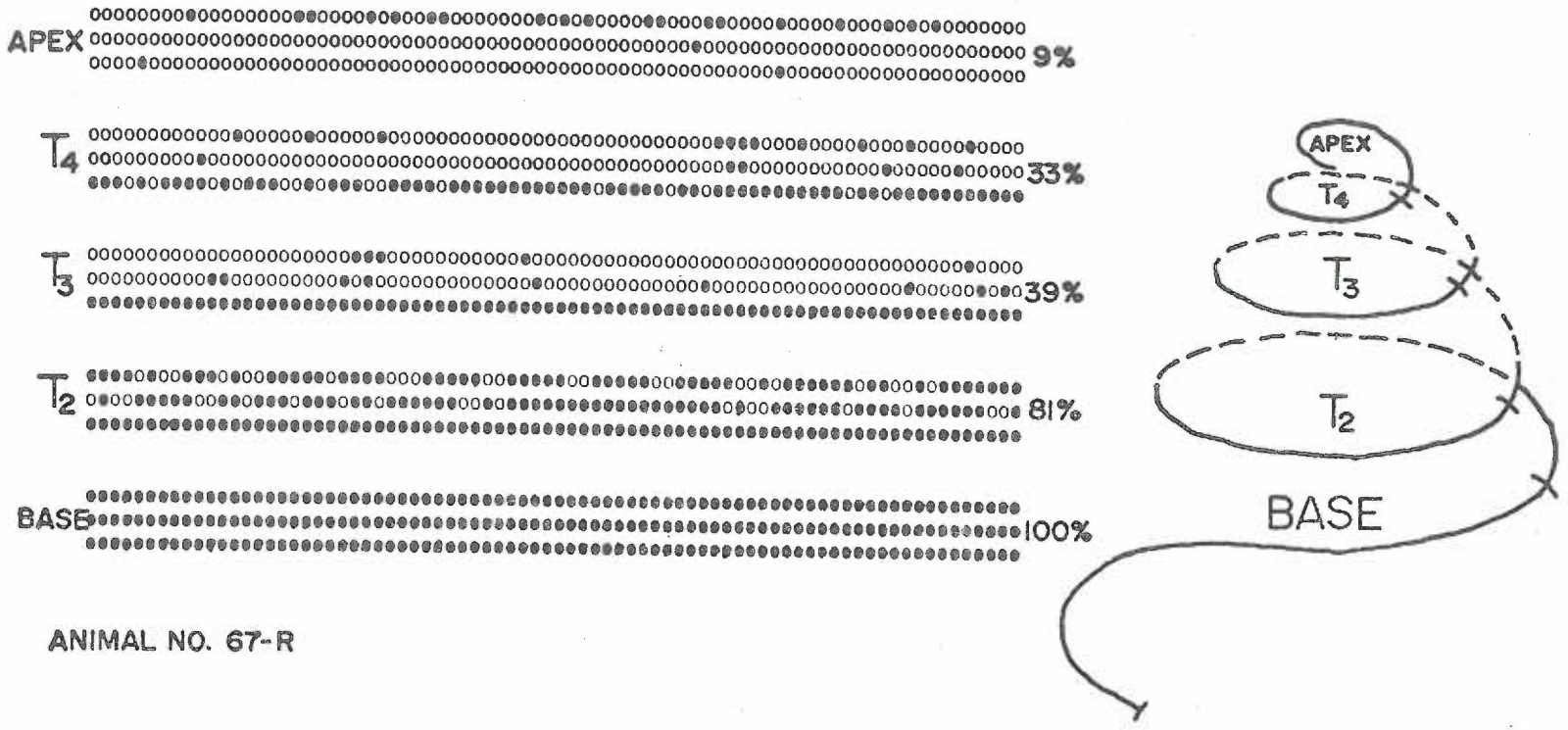
Immediately following the last electrophysiological and sound measurement, each animal was prepared for histological examination of the cochlea using the surface preparation technique (Engstrom, Ades & Andersson, 1966).

While still anesthetized the animal was decapitated, the temporal bones quickly removed and the cochleas perfused with Dalton's solution containing 1% OsO_4 as a fixative for 5 minutes. The temporal bones were then allowed to remain in the fixative for an additional 2 hours. The cochleas were then washed in normal saline and sequentially dehydrated in 35%, 50% and finally 70% ethanol.

With visualization through an operating microscope, the entire organ of Corti was dissected free from the osseous spiral laminae and mounted by turns in glycerine on glass slides. Using phase contrast microscopy, counts were made of the number of missing OHC's in representative lengths of 80 OHC segments at the base, turn 2, turn 3, turn 4 and at the apex of the cochlea. The approximate location of these sites within the cochlea may be seen in Figure 5. This figure shows a sample "cochleogram" which was constructed for each ear showing the numbers of missing cells. From these counts the percentage of missing OHC's was easily computed for each site in each ear. Although precise counts were not made of missing IHC's in the cochlea, the approximate degree of damage to these cells was also noted on the record.

FIGURE 5.

A sample "cochleogram" used for recording the number of missing OHC's from specific areas within the cochlea. Note the relative positions and lengths of segments of the cochlea at the base, turn 2, turn 3, turn 4 and the apex. The three rows of circles for each site indicate the three rows of OHC's in 80 OHC lengths. Percentages indicate the relative number of OHC's that were found missing at that location.


APEX
9%
T4
33%
T3
39%
T2
81%
BASE
100%
ANIMAL NO. 67-R
APEX
T4
T3
T2
BASE

VIII. Group Attrition and Analysis of the Data:

Twenty-nine of the original thirty-two animals survived the experimental procedure. One animal from the Combination group and one animal from the Neomycin-alone group expired during the drug and noise exposure period. Although autopsy was not performed, death was presumed to have occurred due to respiratory arrest induced by the neomycin. One animal from the Noise-alone group expired before completion of the electrophysiological measures and was consequently dropped from the study (Appendix A).

Statistical analysis of the data was performed using each ear of each animal as an independent subject. In the three groups in which an animal was lost from each, the group N was 14 ears. The Control group, in which all animals survived, had an N of 16 ears. In order to facilitate statistical analysis by having equal numbers of animals in each group, the two median ears in each measure of cochlear integrity from the Control group were dropped from the analysis.

Statistical analysis of the data was performed using the analysis of variance. Computations were made on a Wang (Model 720) minicomputer. For the 1 μv isopotential functions the analysis involved a 4 X 2 design in which the first factor was Treatment (Noise-alone, Neomycin-alone, Combination and Control) and the second factor was Frequency (mean isopotential values for each ear below 5 kHz vs. those 5 kHz and above).

The analysis of variance for the intensity function data involved two separate 4 X 1 designs in each of which the first factor was Treatment and the second factor was the frequency at which the measures were taken; 1 kHz or 10 kHz. The measures used for this

analysis were the maximum electrical output each ear was able to attain at the specified frequency.

For the histological measures the analysis again involved a 4 X 2 design in which the first factor was Treatment. The second factor in this measure was Location (mean OHC losses for each ear at the base + turn 2 vs. those turn 3 and above) of damage within the cochlea. In each case described above the Scheffé test (Downie & Heath, 1974) followed the analysis of variance.

RESULTS

The results of this study have confirmed an interaction between neomycin and acoustic overstimulation in terms of toxic effects on the ear. The interaction has been identified both electrophysiologically and histologically. A clear and consistent exacerbation of damage was seen when the Combination group was compared with the Noise-alone and Neomycin-alone groups. The extent of the damage with regard to both electrophysiological and histological measures was far greater in the Combination group than the sum of effects due to each agent when given alone.

The correspondence between the electrophysiological and histological measures for each group of animals was good. That is, the extent and the site of OHC loss agreed well with the severity and nature of the electrophysiological depression.

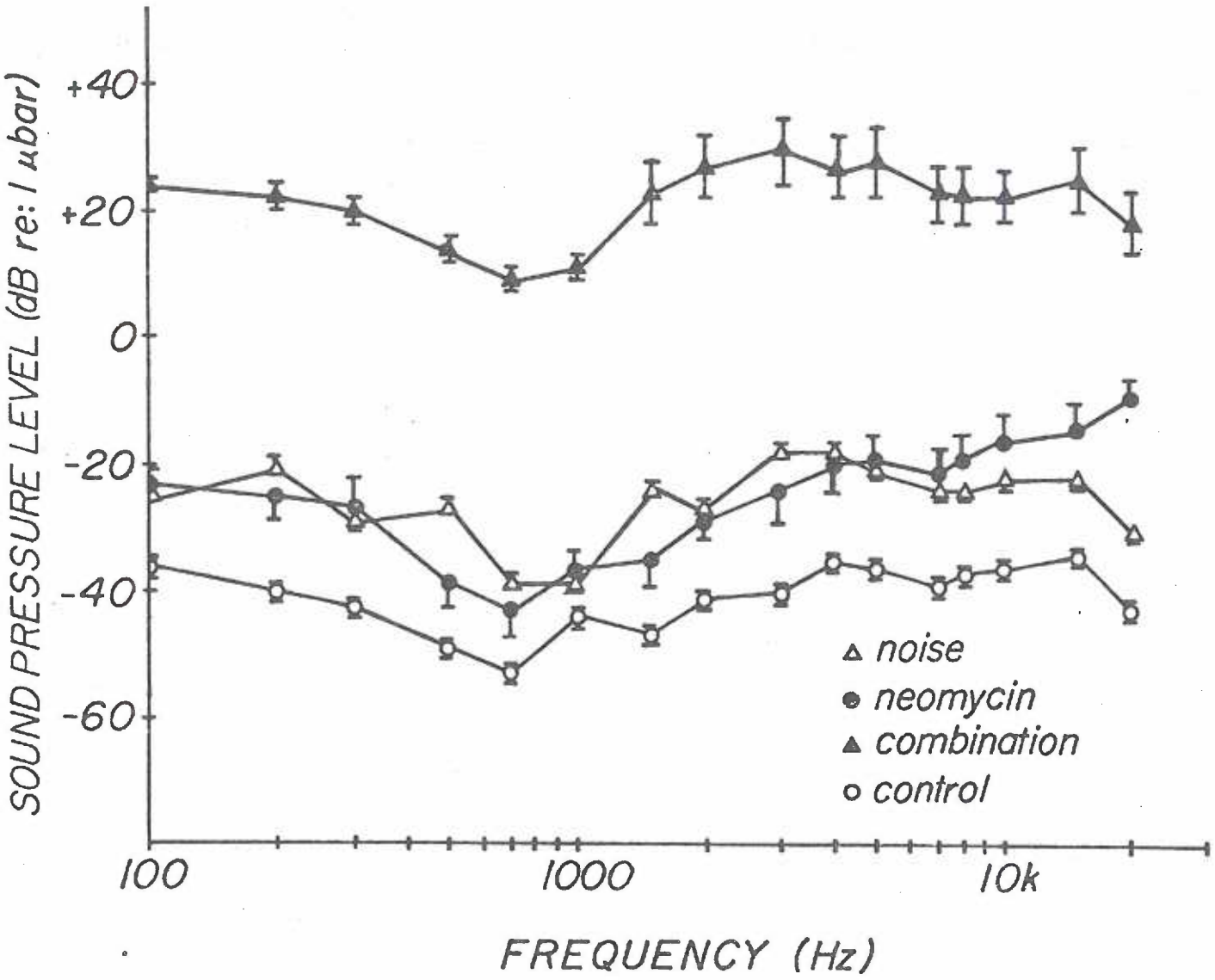
I. Electrophysiological Findings:

The 1 μv isopotential functions for each of the four treatment groups within the range of frequencies from 100 Hz through 20 kHz are shown in Figure 6. Each point represents the mean sound level required at each frequency to elicit a cochlear potential of 1 μv (RMS). The sound pressure required to attain this electrical output, plotted on the ordinate, is inversely proportional to the sensitivity of the ear. Thus the extent of cochlear damage is represented by the height of each curve above the Control group curve at the bottom of the figure.

The group that received neomycin in combination with 115 dB noise is represented by the 1 μv isopotential function at the top of

FIGURE 6.

Averaged 1 μv isopotential functions of the AC cochlear potential obtained from all ears of four groups of animals. Vertical bars represent ± 1 standard error of the mean. Major losses may be seen in the group which received loud noise in combination with neomycin at top of figure. Exposure to noise alone or to neomycin alone resulted in much less damage. Control group may be seen at the bottom of figure.


SOUND PRESSURE LEVEL (dB re: 1 μbar)
+40
+20
0
-20
-40
-60
100
1000
10k
FREQUENCY (Hz)
noise
neomycin
combination
control

the figure. Below this curve, and showing approximately equivalent damage, are the Noise-alone and Neomycin-alone groups. Decreased sensitivity in the Noise-alone and Neomycin-alone groups, while substantial relative to the Control group, is modest in comparison with the Combination group.

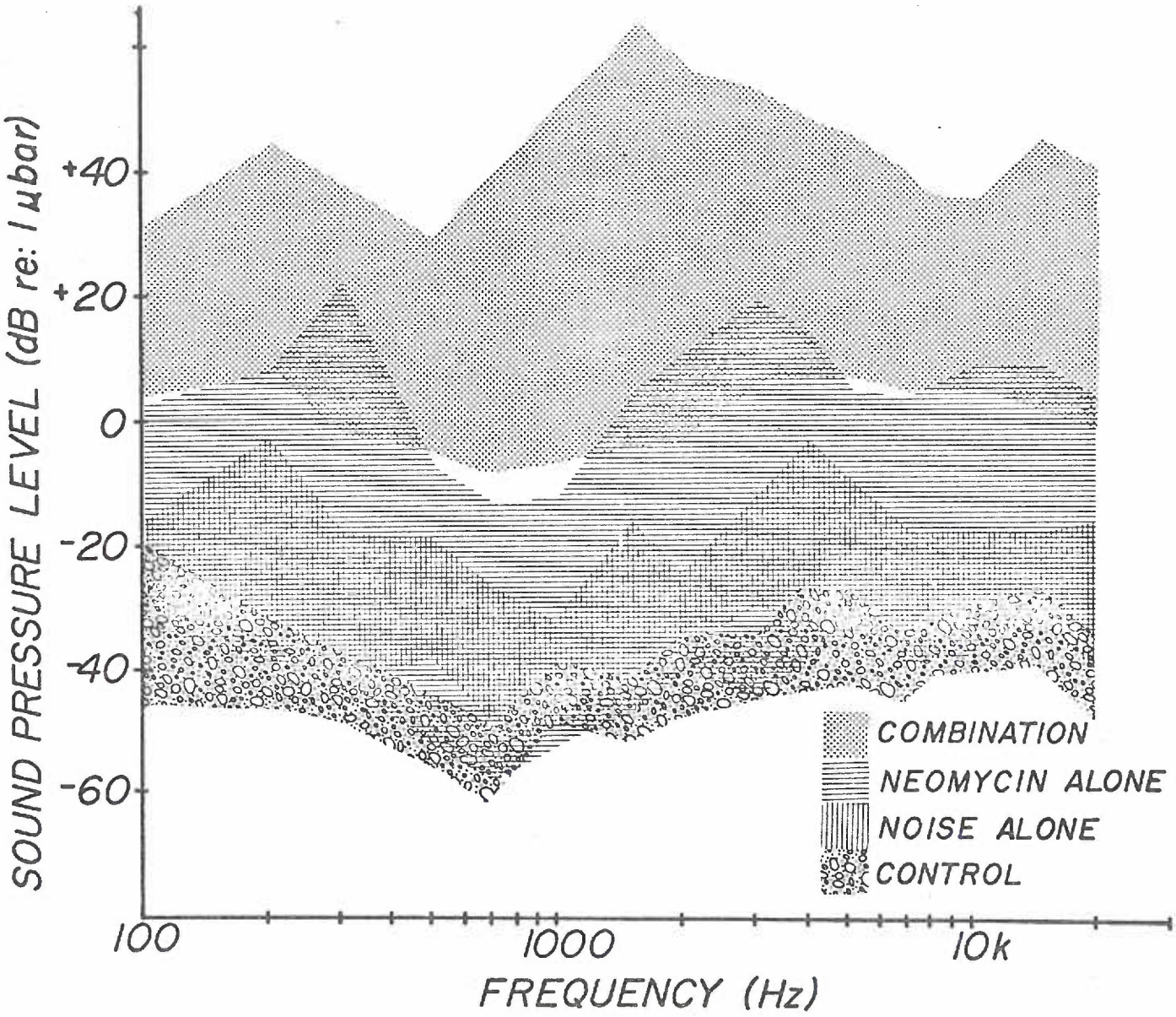
The decrease in sensitivity seen in the Combination group (averaged across all frequencies relative to Control group levels) amounted to 62 dB. For the Noise-alone and Neomycin-alone groups the averaged losses relative to the Control group were only 16 dB and 17 dB respectively. If the sum of the losses due to the individual agents is computed, the 33 dB depression of sensitivity which is calculated is obviously well below the 62 dB depression found for the Combination group.

The analysis of variance applied to the 1 μv isopotential values demonstrated a significant effect due to Treatment, $F_{(3,52)} = 146.02$, $p < .001$, as well as a significant effect due to Frequency, $F_{(1,52)} = 31.66$, $p < .05$ (Appendix B). A significant interaction term (Treatment X Frequency) was identified ($p < .01$). The Scheffé test, applied to Treatment effects indicated that the Control group was significantly different from the Combination group ($p < .001$), from the Neomycin-alone group ($p < .01$), and from the Noise-alone group ($p < .05$). The Combination group was significantly different from the Noise-alone group ($p < .001$) and from the Neomycin-alone group ($p < .001$). The Noise-alone and Neomycin-alone groups were not significantly different.

The Scheffé test applied to Frequency indicated that the only significant difference due to this factor occurred in the

FIGURE 7.

Complete range of data points of the 1 μv isopotential function of the AC cochlear potential obtained from four groups of animals. Wide variability may be seen in the group which received loud noise in combination with neomycin (dotted area) and in the group which received neomycin alone (horizontal lines). Less variability is evident in the group which received noise alone (vertical lines) and in the Control group (stippled area).


SOUND PRESSURE LEVEL (dB re: 1 μbar)
+40
+20
0
-20
-40
-60
100
1000
10k
FREQUENCY (Hz)
COMBINATION
NEOMYCIN ALONE
NOISE ALONE
CONTROL

Neomycin-alone group ($p < .001$). In this group there was significantly more damage in the frequency range from 5 kHz to 20 kHz than at 5 kHz and below.

The complete range of data points for each of the four treatment groups is shown in Figure 7. It should be noted that the range for the Neomycin-alone group completely overlaps that of the Noise-alone group and that many of the values in both of these groups fall within the Control range. There is also partial overlap of Neomycin-alone group values on the Combination group values. The relatively wide range of values in the Neomycin-alone and Combination group contrasts sharply with the more narrow range of values in the Noise-alone and Control groups. It should be noted that the relatively greater variability in the two groups which received neomycin as a treatment is consistent throughout all the measures to be presented below.

A further finding concerns the variability in Combination group values. It may be seen that variability in this group diverges sharply from values below 1 kHz to the values above this frequency as shown by the standard errors of the mean in Figure 6. The narrow range of values below 1 kHz and the wide range of values above this frequency is not seen in any of the other three experimental groups.

The AC cochlear potentials resulting from a sound stimulus of increasing intensity, the intensity functions, are shown for the four treatment groups at 1 kHz in Figure 8 and at 10 kHz in Figure 9. These values represent the means of all ears within each treatment group at each point.

Similar results were obtained at both 1 kHz and at 10 kHz. The major finding was again that the Combination group values were

FIGURE 8.

Intensity functions of the AC cochlear potential at 1 kHz. Each curve represents the mean of all ears within each treatment group. Vertical bars represent ± 1 standard error of the mean.

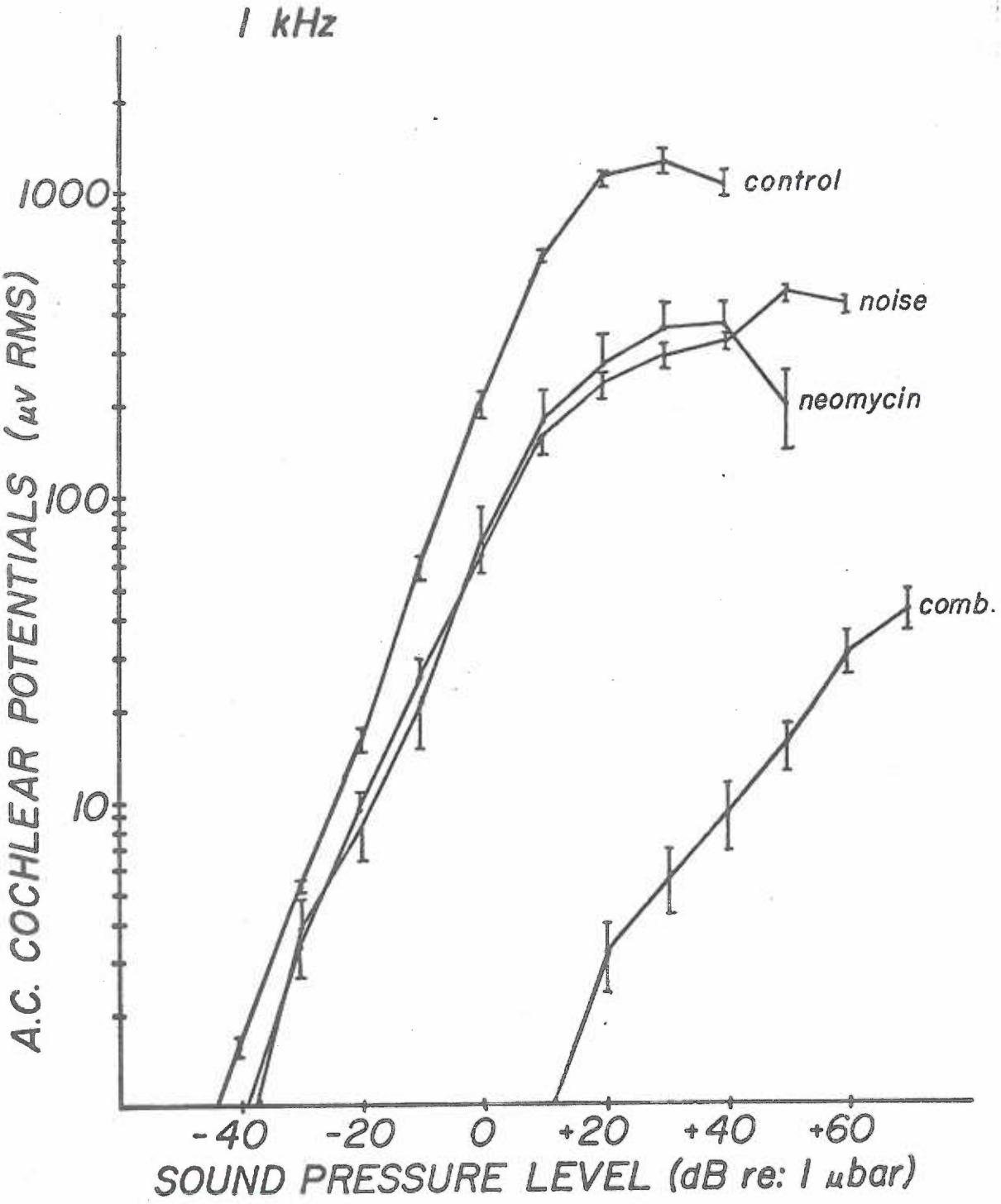
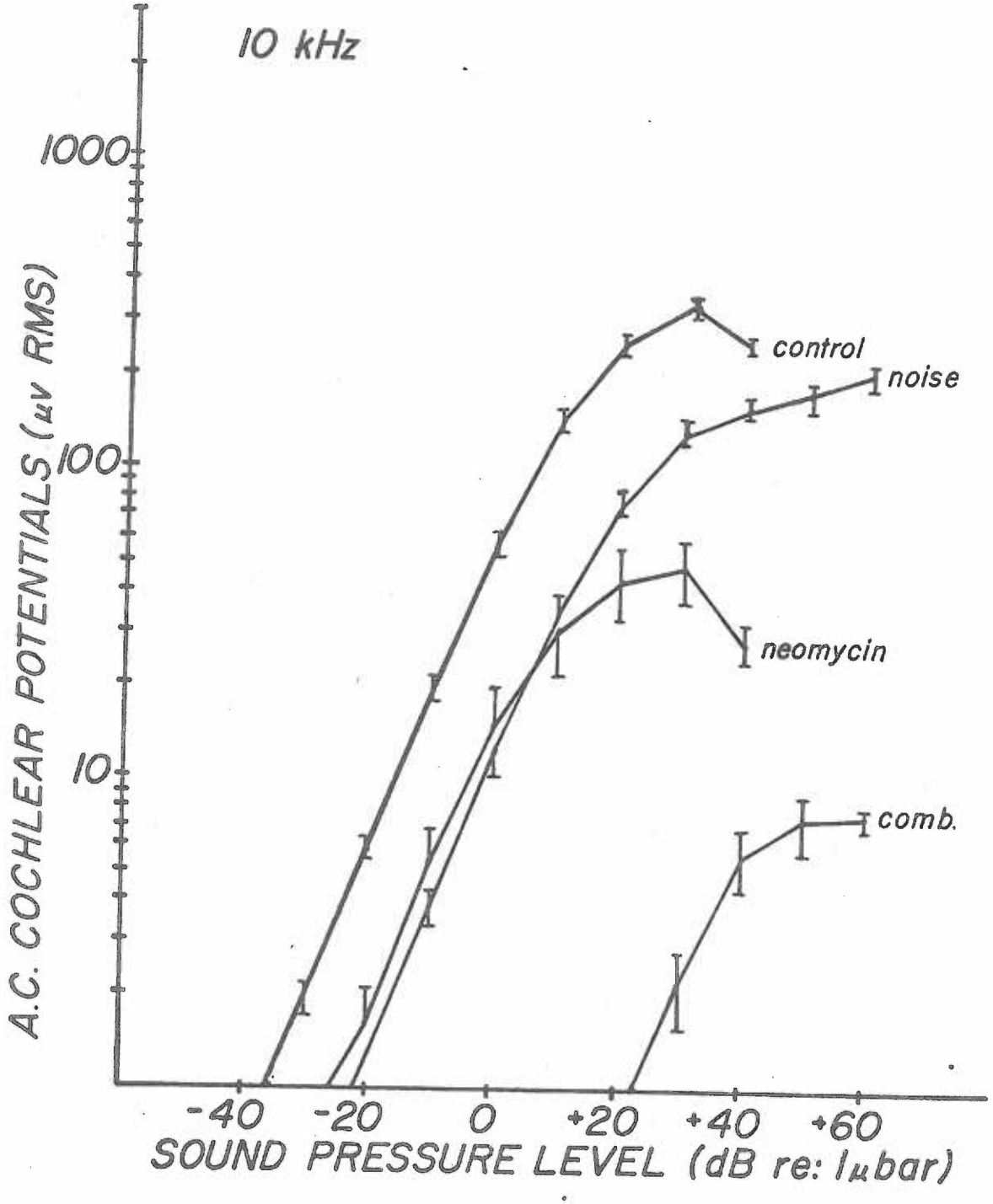

1 kHz
1000
100
10
A.C. COCHLEAR POTENTIALS (μv RMS)
control
noise
neomycin
comb.
-40
-20
0
+20
+40
+60
SOUND PRESSURE LEVEL (dB re: 1 μbar)

FIGURE 9.

Intensity functions of the AC cochlear potential at 10 kHz. Each curve represents the mean of all ears within each treatment group. Vertical bars represent ± 1 standard error of the mean.


10 kHz
1000
100
10
A.C. COCHLEAR POTENTIALS (μv RMS)
control
noise
neomycin
comb.
-40
-20
0
+20
+40
+60
SOUND PRESSURE LEVEL (dB re: 1μbar)

severely depressed relative to Control, Noise-alone and Neomycin-alone group values. Intensity functions for the Combination group were far to the right on the abcissa, indicating losses in sensitivity, and were substantially depressed in height, indicating dramatic losses in the output capability of the ears.

At 1 kHz the single agent groups showed very similar intensity functions with little or no loss in sensitivity relative to the Control group. In terms of output capability, however, substantial losses were found relative to the Controls. It should be noted that at 1 kHz the maximum outputs of the single agent groups were very close to one another.

At 10 kHz the single agent groups showed more definite losses in sensitivity relative to the controls. Additionally, the maximum outputs in these two groups differed markedly. Much more depression was evident in the Neomycin-alone group at 10 kHz than at 1 kHz relative to both Control and Noise-alone group values. This finding was in keeping with the changes in the Neomycin-alone group's 1 μv isopotential function at frequencies above 5 kHz.

A summary of the averaged maximum outputs for each group may be seen in Table I. Separate analysis of variance at each frequency indicated significant differences between means at both 1 kHz, $F_{(3,52)} = 60.78$, $p < .001$, and at 10 kHz, $F_{(3,52)} = 79.65$, $p < .001$ (Appendix C). The Scheffé test for 1 kHz showed the Control group to be significantly different from all other groups ($p < .001$), the Combination group to be significantly different from the single agent groups ($p < .01$), and no difference to exist between the maximum output of the Noise-alone and Neomycin-alone groups. At 10 kHz,

TABLE I.

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

	AVERAGED MAXIMUM OUTPUT	
GROUP	1 kHz	10 kHz
CONTROL	1264μV	318μV
NOISE-ALONE	404μV	185μV
NEOMYCIN-ALONE	377μV	51μV
COMBINATION	24μV	9μV

the Scheffé test revealed all means to be significantly different including Noise-alone vs. Neomycin-alone ($p < .001$) with the exception of Neomycin-alone vs. Combination group means which were not significantly different.

Thus, as could be predicted by the 1 μv isopotential functions, at 1 kHz all groups differed from one another excepting the Noise-alone and Neomycin-alone groups. At 10 kHz however, the increased damage in the higher frequencies due to neomycin rendered the Neomycin-alone group nearly as depressed as the Combination group. As with the 1 μv isopotential functions, variability within the two groups which were exposed to neomycin was greater than that in the Control or Noise-alone groups. This comparison may be made by examination of the standard errors of Figure 8 and Figure 9.

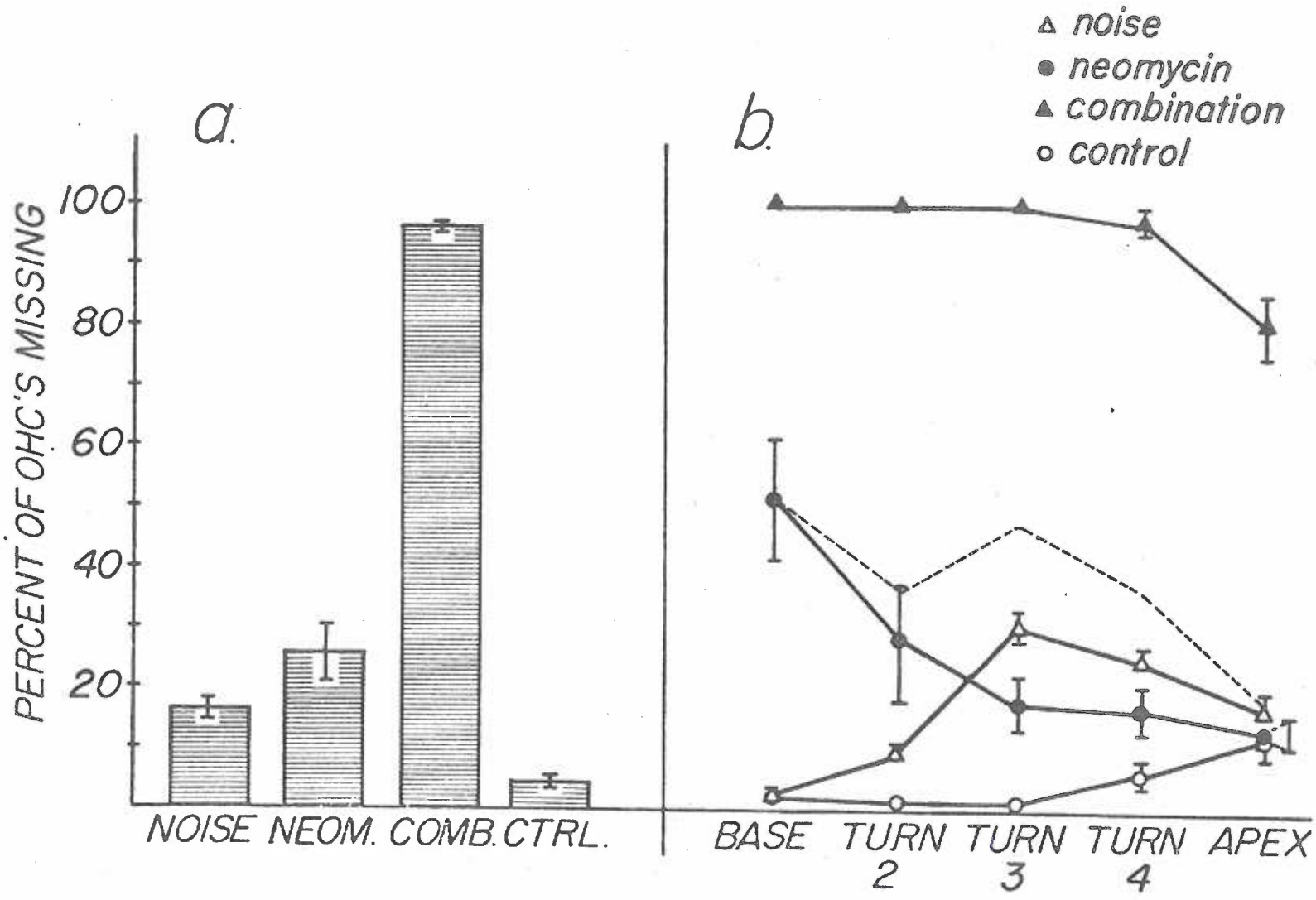
II. Histological Findings:

Total OHC losses expressed as percentage values relative to the total number of OHC's that would exist in an equivalent segment of undamaged organ of Corti are shown in Figure 10(a). These total OHC losses for each group are shown as a function of cochlear location in Figure 10(b).

Again, an interaction effect is clearly visible in the dramatic augmentation of damage due to the combination of noise with neomycin relative to the damage due to these agents given alone. The 97% OHC losses in the Combination group graphically reflect the large depression of the electrophysiological measures found in this group and indicates ears that were operating at near total incapacity. Although IHC losses are not represented in Figure 10, they were noted

FIGURE 10.

Results of outer hair cell counts expressed as a percentage of the total number that would exist in an equivalent segment of undamaged organ of Corti. Counts averaged over all areas of the cochlea are shown for each group of animals in (a); these counts are represented as a function of location in the cochlea in (b). Vertical bars represent $\pm$ 1 standard error of the mean. The dashed line in (b) represents the magnitude of the effects that might be predicted to result from the simple addition of the effects of noise and the effects of neomycin (where dash = Noise-alone value + Neomycin-alone value - Control value).


a.
b.
noise
neomycin
combination
control
PERCENT OF OHC'S MISSING
100
80
60
40
20
NOISE
NEOM.
COMB.
CTRL.
BASE
TURN 2
TURN 3
TURN 4
APEX

to be completely absent in most of the ears in the Combination group.

As would be predicted, more modest OHC losses were associated with moderate electrophysiological depression in the Neomycin-alone and Noise-alone groups. The 27% OHC loss in the Neomycin-alone group was consistent with the 17 dB depression of the 1 μv isopotential function for this group. Similarly the 16% loss of OHC's in the Noise-alone group corresponded with a 16 dB depression of the 1 μv isopotential function. Although the Control group showed 3% of OHC's to be missing, this latter finding is considered normal for the guinea pig cochlea.

Inspection of Figure 10(b) reveals that 100% of the OHC's from the base through turn 3 were missing in the Combination group. The slight sparing of cells at turn 4 and at the apex may have accounted for the slight functional capacity of these ears. Consistent with the damage known to result from the aminoglycoside antibiotics, neomycin when given alone exerted most of its effect at the base and turn 2 of the cochlea. By contrast, damage due to the broad band noise given alone was maximal within turn three. It should be noted that the slight absence of OHC's in the Control group is due to missing cells in turn 4 and at the apex. Once more, variability in the Neomycin-alone group was larger than that in the Noise-alone or Control groups. Variability in the Combination group appeared to have been limited by ceiling effects.

Analysis of variance applied to the OHC losses for each ear revealed highly significant differences between groups due to Treatment, $F_{(3,52)} = 168.02$, $p < .001$. Although no significant difference due to Location was identified, a highly significant

interaction term, $F_{(3,52)} = 15.48$, $p < .001$, was identified indicating that location effects may have been hidden in the analysis (Appendix D).

The Scheffe test applied to Treatment effects indicated that the Combination group was significantly different from the Noise-alone, Neomycin-alone and Control groups ($p < .001$), and that while the Control group differed significantly from the Neomycin-alone group ($p < .01$) it did not differ from the Noise-alone group. The Noise-alone and Neomycin-alone groups were not significantly different.

With respect to Location effects, the Scheffe test indicated significant differences within both the Noise-alone and Neomycin-alone groups ($p < .01$). Visual inspection of Figure 10(b) shows these significant differences with respect to Location to be due to the increased damage near the base due to neomycin when given alone, and increased damage at turn 3 and above due to noise when given alone. The Control group and the Combination group showed no differences with respect to location although it seems likely that ceiling effects may have confounded the latter comparison.

Within every cochlear turn, the number of missing OHC's in the Combination group far exceeded the number missing in the Control, Noise-alone and Neomycin-alone groups combined. The magnitude of the effects that might be predicted to result from the simple addition of the effects of noise and the effects of neomycin is indicated by the dashed line in Figure 10(b) (where dash = Noise-alone value + Neomycin-alone value - Control value). It should be noted that the damage in the Combination group is nearly double that indicated by the dashed line throughout the cochlea.

DISCUSSION

The data have indicated that there is an ototoxic interaction when neomycin is administered concurrently with excessive acoustic stimulation. That is, the magnitude of the damage seen when noise was combined with neomycin was far greater than the simple addition of damage due to noise alone and to neomycin alone. This comparison was evident in both the electrophysiological and histological measures. A review of the data, a discussion of the possible mechanisms underlying the interaction and a discussion of the validity of the interaction follows.

I. The Interaction:

The dramatic augmentation of damage due to the combination of noise with neomycin in electrophysiological measures was demonstrated by the 1 μv isopotential functions. There were significant differences between means not only when the Control group was compared to the Noise-alone and Neomycin-alone groups, but a further significant difference between each of these two groups and the group which received the noise in combination with neomycin. In the 1 μv isopotential functions the mean depression over all frequencies (relative to Control group levels) was 16 dB for the Noise-alone group. The mean depression in the Neomycin-alone group amounted to 17 dB. The sum of these figures, 33 dB, while an imaginary number, theoretically predicts the additive sum of damages which might have resulted from administration of these two agents. Indeed, if exposure to noise and exposure to neomycin were given at widely spaced intervals, that may have been the resulting loss. Further

work may establish this prediction. With concurrent exposure, the loss of 62 dB that was attained is well in excess of the prediction on the basis of simple addition, and implies an interaction.

Neomycin when given alone led to significantly more damage in the high frequencies (above 5 kHz) although overall, the damage due to neomycin given alone was not different from that due to noise alone. This finding is in direct contrast to the findings of Jauhiainen et al (1972) in which neomycin when given alone led to consistently more damage than did noise alone at test frequencies below 4 kHz. As the dosage levels of the drug were the same in both studies, a possible explanation for this discrepancy may be the 30-40 day post-exposure interval employed here, as compared to the 14 day interval employed in the study by Jauhiainen et al.

What might be the relation of the electrophysiological data to human clinical observations? In human audiometric screening, a drop in sensitivity to pure tones is indicated as a hearing impairment only if in excess of 20 dB from "normal" hearing. Losses less than this are not considered significant unless compared to a previous audiogram. While the 1 μv isopotential functions are not a measure of the hearing threshold of the guinea pig, they do represent a sensitivity measure to a low level (in undamaged ears) of stimulation. Consequently a 16 to 17 dB loss if it occurred in humans might not be picked up in normal screening as a significant deviation from normal hearing threshold. A 62 dB loss would be considered a serious impairment. The point is that losses of a lesser nature may go undetected until an interaction rendered them highly significant.

The losses of OHC's in the Noise-alone group (16%), in the Neomycin-alone group (27%) and those found missing in the Control group (3%) when added are again far less than the losses in the Combination group (97%). If one were to predict the amount of destruction in an animal exposed to both of these agents on the basis of simple addition, a loss of 47% of OHC's would be obtained. The 97% losses found in the Combination group are far beyond this and represent ears that are operating at minimal capacity. The loss of OHC's in this group might have been even higher had ceiling effects not limited the counts from sites in the base through turn 3 of the cochlea.

An examination of the location of the greatest OHC losses in the Neomycin-alone group shows the majority of these cells to have been destroyed in the base and turn 2 of the cochlea. In contrast, the losses in the Noise-alone group were maximal within turn 3 of the cochlea. This latter finding is again in contrast with the OHC losses found by Jauhiainen et al (1972) resulting from exposure to an octave band of noise centered at 8 kHz. In that investigation, the maximal OHC losses from exposure to noise were found within turn 2 of the cochlea. The differences between the two findings may be attributable to differences in the type of noise used for sound exposure (octave band vs. broad band) or to differences in experimental procedures.

The finding that damage due to neomycin was greater in the high frequencies than in the low frequencies was significant at the $p < .001$ level. This is consistent with the characteristic damage due to the aminoglycoside antibiotics, usually reported in terms of

hearing thresholds. The selective depression of high frequency hearing was also evident in terms of the maximal output capabilities of the ears when comparing the intensity functions at 1 kHz with those at 10 kHz. At 1 kHz, the intensity functions for both the Noise-alone and the Neomycin-alone groups were close to control values and were not different from one another. At 10 kHz however, the Neomycin-alone curve is much more depressed than the Noise-alone curve and the averaged maximal output in the former group is not different from that in the Combination group.

II. Variability Within the Different Measures:

It is interesting to note that the variability within each group was consistent from measure to measure. That is, the groups showing the largest variability in electrophysiological measures, the Neomycin-alone and Combination groups, also showed large variability in the histological measurements. Conversely, the Noise-alone and the Control groups showed relatively small variability in all measures. There were some exceptions to these findings that deserve some comment.

The low variability in measures of missing OHC's in the Combination group at all locations below turn 4 is attributable to ceiling effects. Since no more than 100% of the OHC's may be missing, the ears in this group would be expected to show very similar levels of the 1 μv isopotential function with consequent low variability. Examination of the 1 μv isopotential function for this group (Figure 6) reveals a biphasic shift in degree of variability from the low to the higher frequencies. A substantial amount of variability

exists at frequencies above 1 kHz in the Combination group isopotential function, which is roughly equivalent to that seen in the Neomycin-alone group. At frequencies below 1 kHz the variability in the Combination group is quite small, roughly equivalent to that found in the Noise-alone group.

Without a precise knowledge of the curve which represents the degree of depression of electrophysiological measures resulting from specific amounts of OHC and IHC damage, it is difficult to predict the ceiling level of the 1 μv isopotential function. It should be possible to predict that level by examination of the 1 μv isopotential functions from another study using similar testing procedures, in which a high degree of hair cell destruction was reported.

In the investigation of the ototoxic interaction between kanamycin and ethacrynic acid, West, Brummett and Himes (1973) report the 1 μv isopotential functions from two guinea pigs showing essentially complete OHC and IHC loss 30 days following exposure to combinations of these two agents. It may be suggested that these isopotential functions represent the ceiling level of this measure in the guinea pig using a very similar test procedure as employed in this study.

When the data of West et al are compared with the 1 μv isopotential functions of the Combination group, a great deal of similarity may be seen. This similarity is especially pronounced at frequencies from 100 Hz through 2 kHz, in which range the values from these two studies are almost identical. At frequencies above 2 kHz however, the 1 μv isopotential functions of West et al show much further losses in sensitivity (on the order of 20 dB) when compared to the Combination group levels of Figure 6.

It may be suggested that the level of the 1 μv isopotential function in the group that received the neomycin in combination with loud noise represents the ceiling limit of this measure at frequencies below 2 kHz but not above. If this were the case, the change in variability from the low to the high frequencies observed in this measure from the Combination group might be explained on the basis of electrophysiological ceiling effects.

On the basis of the 100% loss of OHC's within the base and the relative sparing of OHC's at the apex observed in the Combination group it would be predicted that the ceiling limit, if any, would first be reached in the high frequencies of the 1 μv isopotential function. Additionally, the well known proclivity for damage in these frequencies due to the aminoglycoside antibiotics and to loud noise would have led to a similar prediction. That this was not observed implies that further investigation is required.

III. Speculation of the Mechanism of the Interaction:

The possible mechanisms underlying the interaction between noise and neomycin in the ear remain to be elucidated. It may be that the interaction is not gained by a direct action of both agents at the same site, that is, at the hair cells. It is conceivable that noise exposure might lead to alterations in the physiology of the animal with consequent alterations in the pharmacodynamics of neomycin. Noise induced changes may affect the distribution or elimination of neomycin, either systemically or localized to the ear.

Subsequent investigations of drug distribution in noise exposed guinea pigs has discouraged this hypothesis. No differences in drug

levels in either plasma or perilymph has been identified in the 24 hour period following a single injection between animals subsequently exposed to a high versus a low sound environment. This has been investigated for both neomycin and kanamycin. While these findings do not rule out the hypothesis of an interaction based on differences in drug distribution, it makes it unlikely that increased damage in animals exposed to a combination of agents is due to changes in the concentration of the drug either systemically or localized to the ear.

The morphological damage resulting from exposure to both noise and to neomycin have much in common. Effects first occur in the OHC's. Effects are commonly first noted in the high frequencies. Disarrangements of cellular contents and membranes are followed by cell swelling and lysis. The common elements of destruction due to the two agents and the lack of significant differences in drug distribution lead one to predict that the interaction between noise and neomycin is occurring at or within the hair cells themselves.

The action of the aminoglycoside antibiotics is generally considered to occur due to their ability to bind to the bacterial 30S ribosome. As eucaryotic ribosomes are composed of different subunits they are presumably not affected, except in the kidney and the inner ear. The damage to these mammalian tissues is not known to occur due to interference with protein synthesis. Rapid and reversible supression of the electrical output of the lateral line organ of fish has been observed to occur within 5 minutes after infusion of streptomycin (Wersall & Flock, 1964), or of neomycin (Nuttall, Marques, Sterhorst & Schacht, 1975) into the inner ear. The time course of these changes is not generally consistent with the

interference of protein synthesis as a primary cause of damage.

Damage might occur as a result of an interference with the metabolic capacity of the cells of the ear by action of the antibiotics on the mitochondrial 30S ribosome of the mammalian cell. Increased energy requirements due to the noise exposure conceivably might compound this injury. Decreases in cochlear blood flow due to extremely intense sound (Pearlman & Kimura, 1961; Hawkins, 1971) may increase cellular dependence on mitochondrial metabolism.

Some evidence now exists that the aminoglycoside antibiotics do not cross cell membranes as easily as once thought, and may exert primary effects on cell membranes leading to alterations in permeability and structure of cells. Schacht (1976) has shown that neomycin will bind to and interfere with the turnover of phosphinositide lipids in homogenates of the organ of Corti. Interference with the turnover of these lipids, which provide structural support for the cell membrane, may cause rapid shifts in the permeability of these cells. Such permeability shifts would necessarily lead to osmolarity changes and the diffusion of fluids between the cell and its environment. Diffusion of fluids from the outside to the inside of the hair cells may lead to the swelling of these cells observed in response to the aminoglycoside antibiotics. It is also conceivable that this swelling might render these cells more susceptible to mechanical trauma than they would otherwise be. Thus, cells swollen by neomycin might return to normal size over time unless ruptured first by exposure to loud noise. Additionally the swelling observed in response to excessive noise, when added to the swelling of cells due to the antibiotics, may lead to increased incidence of cell lysis.

These possibilities remain speculative until the direct observation of the interaction becomes possible.

IV. A Note on Terminology:

Webster's Third International Dictionary (1966) defines an interaction as a "mutual or reciprocal action or influence." There is no reference to the direction that interaction may take. On the other hand, the term potentiation is defined as "to augment (as the pharmacological effect of a drug) with a second drug so that the response produced is greater than the predictable additive effect." The operative words in this latter definition are: "the predictable additive effect."

Despite the previous reports of the interaction between noise and the aminoglycoside antibiotics as a potentiation of damage (Gannon & Tso, 1969; Dayal et al, 1971; Dayal et al, 1975; Hawkins et al, 1975; Marques et al, 1975), care has been taken in this report to avoid the use of the term. In the strict pharmacological sense, a potentiation cannot be identified until the dose-response curve for each of the agents is known and the precise points on that curve identified for each exposure level. A plot of a response as a percent of maximum on the ordinate with a plot of the log dose on the abcissa will reveal, for most pharmacological agents, an "S" shaped sigmoid curve. The curve has slightly sloping asymptotes at both the top and the bottom with a steep midsection. It is in this steep midsection that small increases in the dose of an agent will lead to large increases in the response. For conceptual purposes, let us suppose that damage due to the neomycin alone or to the noise

alone places the animals' response at the beginning of the steep midsection of the log dose-response curve. Further exposure to each agent, even in small increments may cause large increases in damage not readily predictable from the simple addition of single agent effects. The "predictable additive effect" is then not gained by the simple addition of effects due to each agent alone. The interaction that has been observed here may have produced the actual predictable additive effect. Until the dose-response curve for each individual agent is compared with the dose-response curve for the combination of agents a potentiation cannot be identified.

V. Further Directions of Research and Clinical Implications:

Obviously the first step is the elaboration of the dose response curves described above. Once these curves have been described, the true level of the interaction may be established and the temporal relationships in their administration may be elucidated.

Further work in the area of noise and aminoglycoside antibiotic interactions should be aimed at investigating possible interactions between other of these compounds and noise. Such drugs as kanamycin, gentamicin, tobramycin and amakacin which are of more prevalent clinical use should be the next objects of investigation in terms of possible interactions.

Clinically, great care must be taken to ensure that patients who are exposed to these drugs are kept free from excessive noise exposure. This is also true for the users of preparations containing aminoglycoside antibiotics for topical use. The levels of these agents, even if safe when given alone, may lead to a hearing

loss if administration is concurrent with noise exposure. The noise levels produced by suction pumps, respirators, surgical drills and even banging trays, while safe for the untreated patient, may be harmful for those exposed to some drugs. Individuals who are being treated with an aminoglycoside antibiotic should be frequently monitored for any hearing loss, and if such a loss is identified, different therapy considered.

SUMMARY AND CONCLUSIONS

An investigation was conducted into the possible interaction between neomycin and acoustic overstimulation in the guinea pig cochlea. A dramatic augmentation of damage was identified electrophysiologically and histologically in the cochleas of the animals that received neomycin in combination with loud noise when compared with the damage found in animals that had received either agent alone. From the results of this study it has been concluded:

1. An interaction between neomycin and acoustic overstimulation exists such that the damage due to a combination of these agents may be more than twice the level estimated by simple addition of the effects of each when given alone. This finding confirms and extends the observations of Jauhiainen, Kohonen & Jauhiainen (1972).
2. Exposure to a broad band of noise leads to fairly even depression of the 1 μv isopotential function from 100 Hz through 20 kHz, whereas neomycin given alone leads to increased depression of this measure in the high frequencies. This is probably due to concentration of damage within the base of the cochlea due to neomycin.
3. Exposure to neomycin in combination with noise, or neomycin alone led to more variable results in electrophysiological and histological measures of cochlear integrity than did exposure to noise alone.

REFERENCES

1. Beagley, H.A. Acoustic trauma in the guinea pig. I. Electrophysiology and histology. Acta Otolaryngol., 1965. 60, 437-451.

2. Beard, N.S. Jr., Armentrout, S.A., Weisberger, A.S. Inhibition of mammalian protein synthesis by antibiotics. Pharmacol. Rev., 1969. 21, 213-245.

3. Bekesy, G. von. Experiments in hearing. New York: McGraw-Hill, 1960.

4. Benitez, J.T., Schuknecht, H.F., Brandenburg, J.H. Pathologic changes in human ear after kanamycin. Arch. Otolaryngol., 1962. 75, 192-197.

5. Bohne, B. Mechanism for continued degeneration in the organ of Corti. J. Acoust. Soc. Am., 1974. 55, Suppl. S77 (Abstract).

6. Brummett, R.E. Ototoxic Drugs. Therapeutic Consult., 1973. 5, 15-22.

7. Brummett, R.E., Brown, R. Tobramycin ototoxicity: a second look. Arch Otolaryngol., 1975. 101, 540-543.

8. Brummett, R.E., Harris, R.F., Lindgren, J.A. Detection of ototoxicity from drugs applied topically to the middle ear space. Laryngoscope, 1976. 86, No. 8, 1177-1187.

9. Culler, E.A. In Symposium on tone localization in the cochlea. Ann. Otol. Laryng. Rhin., 1935. 44, 807-813.

10. Dayal, V.S., Kokshanian, A., Mitchell, D.P. Combined effects of noise and kanamycin. Ann. Otolaryngol., 1971. 80, 897-902.

11. Dayal, V.S., Smith, E.L., McCain, G. Cochlear and vestibular gentamicin toxicity. Arch. Otolaryngol., 1974. 100, 338-340.

12. Dayal, V.S., Barek, W.G. Cochlear changes from noise, kanamycin and aging. II. Potentiating effects of noise and kanamycin. Laryngoscope, 1975, 85, Ann. Suppl. No. 1, 8-11.

13. Downie, N.M., Heath, R.W. Basic statistical methods. New York: Harper and Row, 1974.

14. Engstrom, H., Ades, H.W., Andersson, A. Structural pattern of the organ of Corti. Stockholm: Almqvist and Wiksell, 1966.

15. Engstrom, H., Ades, H.W., Bredberg, G. Normal structure of the organ of Corti and the effect of noise induced cochlear damage. In E.W. Walstenholme & J. Knight (Eds.) Sensorineural hearing loss. Baltimore, Maryland: Williams and Wilkens, 1970. pp. 127-156.

16. Falk, S.A. Combined effect of noise and ototoxic drugs. Environ. Health Perspect., 1972. 2, 5-22.

17. Fernandez, C. Dimensions of the guinea pig cochlea. J. Acoust. Soc. Am., 1952. 24, 519-523.

18. Fields, R.L. Neomycin ototoxicity. Arch. Otolaryngol., 1964. 79, 83-86.

19. Fosbroke, J. Practical observations on the pathology and treatment of deafness. Lancet, 1831. 1, 645-648.

20. Fried, M.P., Dudek, S.E., Bohne, B.A. Basal turn cochlear lesions following exposure to low frequency noise. Tr. Am. Acad. Opth. & Otol., 1976. 82, 285-298.

21. Galambos, R., Davis, H. The responses of single auditory nerve fibers to acoustic stimulation. J. Neurophysiol., 1943. 6, 39-58.

22. Gannon, R.P., Tso, S.S. The occult effect of kanamycin on the cochlea. Exerpta Medica, 1969. 189, 98 (Abstract).

23. Gibson, W.S. Deafness due to orally administered neomycin. Arch. Otolaryngol., 1967. 86, 163-165.

24. Goodman, L.S., Gilman, A. (Eds.) The pharmacological basis of therapeutics. New York: MacMillan Co., 1975.

25. Greenwood, D.D. Critical bandwidth and the frequency coordinates at the basilar membrane. J. Acoust. Soc. Am., 1961. 33, 1344-1348.

26. Habermann, J. Über die schwerhorigkeit der kesselschmiede. Arch. Ohrenheilk, 1890. 30, 1.

27. Hammernik, R.P., Henderson, D. The potentiation of noise by other ototraumatic agents. In D. Henderson, R.P. Hammernik, D.S. Dosanjh, J.H. Mills (Eds.) Effects of noise on hearing. New York: Raven Press, 1976. pp. 291-307.

28. Harris, R.F. Ototoxic Drugs. Unpublished Monograph, University of Oregon Health Sciences Center, June, 1972.

29. Hawkins, J.E. Jr., Engstrom, H. Effect of kanamycin on cochlear cytoarchitecture. Acta Otolaryngol., 1964, Suppl. 188, 100-106.

30. Hawkins, J.E. Jr. Biochemical aspects of ototoxicity. In M.M. Paparella (Ed.) Biochemical mechanisms in hearing and deafness. Springfield, Ill: Charles C. Thomas, 1970. pp. 323-339.

31. Hawkins, J.E. Jr. The role of vasoconstriction in noise induced hearing loss. Ann. Otolaryngol., 1971. 80, 903-913.

32. Hawkins, J.E. Jr., Marques, D.M., Clark, C.S. Noise and kanamycin interaction in the guinea pig cochlea. J. Acoust. Soc. Am., 1975. 58, Suppl. No. 1, S89 (Abstract).

33. Helmholtz, H.L.F. von. Sensations of tone. New York: Longmans Green, 1930 (Tr. A.J. Ellis, 5th ed.).

34. Institute of Laboratory Animal Resources, National Research Council. Guide for animal facilities and care. United States Department of Health Education and Welfare, Public Service Publication No. 1024, 1968.

35. Jauhiainen, T., Kohonen, A., Jauhiainen, M. Combined effect of noise and neomycin on the cochlea. Acta Otolaryngol., 1972. 73, 387-390.

36. Kellerhals, B. Acoustic trauma and cochlear microcirculation. Adv. Oto-Rhino Laryngol. (Basel, Switzerland) 1972. 18, 91-168.

37. Lawrence, M., Yantis, P.A. Individual differences in functional recovery and structural repair following overstimulation of the guinea pig ear. Ann. Otol. Rhin. Laryngol., 1957. 66, 595-621.

38. Leach, W. Ototoxicity of neomycin and other antibiotics. J. Laryng., 1962. 76, 774-790.

39. Lim, D.J., Melnick, W. Acoustic damage of the cochlea, a scanning and transmission electron microscope observation. Arch. Otolaryngol., 1971. 94, 294-305.

40. Marques, D.M., Clark, C.S., Hawkins, J.E. Jr. Potentiation of cochlear injury by noise and ototoxic antibiotics in the guinea pig. J. Acoust. Soc. Am., 1975. 57, Suppl. No. 1, pp. S60 (Abstract).

41. Merriam, G., Merriam, C. (Eds.) Webster's third new international dictionary. Chicago, Ill.: The Lakeside Press, 1966.

42. Mitchell, C. The temporary effects of intense sound and sodium salicylate on the electrical activity of the cochlea. Unpublished doctor's dissertation, University of Oregon Medical School, 1973.

43. Nuttall, A.L., Marques, D.B., Sterhorst, E., Schacht, J. Neomycin effects on cochlear microphonics and phospholipid metabolism. J. Acoust. Soc. Am., 1975. 57, Suppl. No. 1, pp. S60 (Abstract).

44. Perlman, H.B., Kimura, R. Cochlear blood flow in acoustic trauma. Acta Otolaryngol., 1961. 54, 99-110.

45. Pittinger, C., Adamson, R. Antibiotic blockade of neuromuscular function. Ann. Rev. Pharmacol., 1972. 12, 169-184.

46. Reddy, J.B., Igaroshi, M. Changes produced by kanamycin. Arch. Otolaryngol., 1962. 76, 146-150.

47. Schacht, J. Biochemistry of neomycin ototoxicity. J. Acoust. Soc. Am., 1976. 59, 940-944.

48. Schatz, A., Bugie, S., Waksman, S.A. Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. Proc. Soc. Exp. Biol. Med., 1944. 57, 244-248.

49. Spoendlin, H. Primary structural changes in the organ of Corti after acoustic overstimulation. Acta Otolaryngol., 1971. 71, 166-176.

50. Spoendlin, H. Anatomical changes following various noise exposures. In D. Henderson, R.P. Hammernik, D.S. Dosanjh, J.H. Mills (Eds.) Effects of noise on hearing. New York: Raven Press, 1976.

51. Stebbins, W.C., Miller, J.M., Johnsson, L., Hawkins, J.E. Jr. Ototoxic hearing loss and cochlear pathology in the monkey. Ann. Otol. Rhin. Laryngol., 1969. 87, 1007-1025.

52. Stockwell, C.W., Ades, H.W., Engstrom, H. Patterns of hair cell damage after intense auditory stimulation. Ann. Otol. Rhin. Laryngol., 1969, 78, 1144-1168.

53. Vernon, J.A., Meikle, M.B. Electrophysiology of the cochlea. In R.F. Thompson, M.M. Patterson (Eds.) Methods in physiological psychology: Vol. I. Recording of bioelectric potentials. New York: Academic Press, 1974.

54. Vernon, J.A., Brummett, R.E., Brown, R. Noise trauma induced in the presence of loop-inhibiting diuretics. Otol. Rhin. Laryngol., 1977. 84, 407-413.

55. Waitzova, D., Kyndand, J., Sobek, K. The toxic effects of neomycin. In M. Harold, Z. Gabriel (Eds.) Antibiotics-advances in research, production and clinical use. London: Butterworths, 1966.

56. Walloch, R.A., Brummett, R.E., Himes, D. Acoustic trauma in two highly inbred strains of guinea pigs. J. Aud. Res., 1976. 16, 89-97.

57. Ward, P.H., Fernandez, C. The ototoxicity of kanamycin in guinea pigs. Ann. Otol. Rhin. Laryngol., 1961. 70, 132-142.

58. Wersall, J., Flock, A. Supression and restoration of the microphonic output from the lateral line organ after local application of streptomycin. Life Sci., 1964. 3, 1151-1155.

59. West, B., Brummett, R.E., Himes, D. Interaction of kanamycin and ethacrynic acid. Arch. Otolaryngol., 1973. 98, 32-38.

60. Wright, C.G. Neural damage in the guinea pig cochlea after noise exposure. A light microscope study. Acta Otolaryngol., 1976. 82, 82-94.

61. Ylikoski, J., Wersall, J., Bjorkroth, B. Correlative studies on the cochlear pathology and hearing loss in guinea pigs after intoxication with ototoxic antibiotics. Acta Otolaryngol., 1974. Suppl. No. 326.

APPENDIX A

TAG NUMBERS, GROUPS, AND WEIGHTS ONE DAY 1, 4, 7, and 30+ FOR ALL ANIMALS

TAG #	GROUP	WEIGHT* DAY 1	WEIGHT DAY 4	WEIGHT DAY 7	DAY SACRIFICED
51	Noise-alone	404	372	384	540
52	Noise-alone	380	380	391	512
53	Combination	192	Died on Day 2----------		
54	Noise-alone	232	245	259	496
55	Combination	280	276	292	415
56	Combination	302	309	300	415
57	Combination	369	388	352	425
58	Noise-alone	354	356	358	546
59	Combination	348	348	350	500
60	Noise-alone	274	281	293	550
61	Combination	248	243	242	441
62	Noise-alone	276	261	267	413*
63	Noise-alone	358	349	348	505
64	Noise-alone	193	192	188	400
65	Combination	385	380	378	497
66	Combination	380	374	373	608
67	Neomycin-alone	330	296	305	467
68	Control	224	216	225	483
69	Neomycin-alone	313	298	287	471
70	Control	201	192	209	376
71	Control	220	224	240	484

*Died during the electrophysiological measurements.

APPENDIX A (continued)

TAG #	GROUP	WEIGHT DAY 1	WEIGHT DAY 4	WEIGHT DAY 7	WEIGHT DAY SACRIFICED
72	Control	235	242	255	450
73	Neomycin-alone	393	396	369	542
74	Control	306	309	326	586
75	Neomycin-alone	309	291	284	434
76	Neomycin-alone	222	231	245	465
77	Control	221	222	221	434
78	Control	382	362	369	515
79	Control	354	353	369	552
80	Neomycin-alone	236	Died on Day 3----------		
81	Neomycin-alone	206	204	216	387
82	Neomycin-alone	361	323	326	576
	MEANS	296	297	301	476

APPENDIX B

ANALYSIS OF VARIANCE FOR 1 μv ISOPOTENTIAL FUNCTIONS

(Two-way, repeated measures on B, Frequency)*

	SS	DF	MS	F	p
BET SUBJ	67404.756	55			
A	60252.576	3	20084.192	146.02	<.001
ERROR	7152.180	52	137.541		
WITH SUBJ	3297.800	56			
B	1043.100	1	1043.100	31.66	< .05
AB	541.673	3	180.557	5.48	< .01
ERROR	1713.026	52	32.942		

GROUPS INVOLVED	SOURCE OF VARIATION	F**	DF	p
Control X Noise-alone	Treatment	12.56	(3,52)	< .05
Control X Neomycin-alone	Treatment	14.85	(3,52)	< .01
Control X Combination	Treatment	196.97	(3,52)	< .001
Neomycin-alone X Noise-alone	Treatment	.09	(3,52)	ns
Neomycin-alone X Combination	Treatment	103.66	(3,52)	< .001
Noise-alone X Combination	Treatment	110.04	(3,52)	< .001
Control X Control	Frequency	7.67	(1,52)	ns
Noise-alone X Noise-alone	Frequency	1.77	(1,52)	ns
Neomycin-alone X Neomycin-alone	Frequency	37.59	(1,52)	< .001
Combination X Combination	Frequency	1.03	(1,52)	ns

* Values were compiled by taking the mean isopotential values for each ear below 5 kHz vs. those 5 kHz and above.

**Result of Scheffé test.

APPENDIX C

ANALYSIS OF VARIANCE FOR MAXIMUM OUTPUTS OF INTENSITY FUNCTIONS (1 kHz)

(One-way, equal groups)

11698265.30570000 = SSB
3335852.10290000 = SSE
15034117.40860000 = SST
3899421.76856000 = A
64151.00197880 = B
60.78504853 = $F\text{-VALUE}_{(3,52)}$ $p < .001$
3.00000000 = k-1
52.00000000 = k(n-1)

GROUPS INVOLVED	SOURCE OF VARIATION	F*	DF	p
Control X Noise-alone	Treatment	83.07	(3,52)	$< .001$
Control X Neomycin-alone	Treatment	85.4	(3,52)	$< .001$
Control X Combination	Treatment	167.7	(3,52)	$< .001$
Neomycin-alone X Noise-alone	Treatment	.01	(3,52)	ns
Neomycin-alone X Combination	Treatment	13.6	(3,52)	$< .01$
Noise-alone X Combination	Treatment	14.69	(3,52)	$< .01$

* Result of Scheffé test.

APPENDIX C (continued)

ANALYSIS OF VARIANCE FOR MAXIMAL OUTPUTS OF INTENSITY FUNCTIONS (10 kHz)

(One-way, equal groups)

813773.78768000 = SSB
177090.88072000 = SSE
990864.66840000 = SST
271257.92922600 = A
3405.59386000 = B
79.65069834 = $F\text{-VALUE}_{(3,52)}$ $p < .001$
3.00000000 = k-1
52.00000000 = k(n-1)

GROUPS INVOLVED	SOURCE OF VARIATION	F*	DF	p
Control X Noise-alone	Treatment	41.4	(3.52)	<.001
Control X Neomycin-alone	Treatment	146.5	(3,52)	<.001
Control X Combination	Treatment	196.2	(3.52)	<.001
Neomycin-alone X Noise-alone	Treatment	32.1	(3,52)	<.001
Neomycin-alone X Combination	Treatment	3.62	(3,52)	ns
Noise-alone X Combination	Treatment	57.3	(3,52)	<.001

* Result of Scheffé test.

APPENDIX D

ANALYSIS OF VARIANCE FOR OUTER HAIR CELL COUNTS

(Two-way, repeated measures on B, Location)*

	SS	DF	MS	F	p
BET SUBJ	160896.334	55			
A	145850.441	3	48616.813	168.02	<.001
ERROR	15045.893	52	289.344		
WITH SUBJ	15456.585	56			
B	97.129	1	97.129	.62	ns
AB	7246.776	3	2415.592	15.48	<.001
ERROR	8112.678	52	156.013		

GROUPS INVOLVED	SOURCE OF VARIATION	F**	DF	p
Control X Noise-alone	Treatment	3.16	(3,52)	ns
Control X Neomycin-alone	Treatment	14.23	(3,52)	<.01
Control X Combination	Treatment	208.52	(3,52)	<.001
Neomycin-alone X Noise-alone	Treatment	3.98	(3,52)	ns
Neomycin-alone X Combination	Treatment	113.78	(3,52)	<.001
Noise-alone X Combination	Treatment	160.34	(3,52)	<.001
Control X Control	Location	1.66	(1,52)	ns
Noise-alone X Noise-alone	Location	15.45	(1,52)	<.01
Neomycin-alone X Neomycin-alone	Location	27.61	(1,52)	<.01
Combination X Combination	Location	2.39	(1,52)	ns

* Values are the mean missing OHC's at the base plus turn 2 vs. those turn 3 and above for each ear.

**Result of Scheffé test.