THE TEMPORARY EFFECTS OF INTENSE SOUND AND SODIUM SALICYLATE ON THE ELECTRICAL ACTIVITY OF THE COCHLEA

BY

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Baseline data of the a. c. cochlear potential and the auditory evoked neural potential (N_1) from as many as seventy-nine guinea pigs are presented for the first time. These electrophysiological measures of cochlear function were used to specify changes induced by both intense sound and sodium salicylate. Both of these agents were found to reduce the amplitude of the auditory evoked neural potential (N_1) while leaving the alternating current cochlear potential unaffected. Further, in the case of intense tones, the effect on N_1 was shown to be frequency specific and was found to be maximal at one half an octave above the exposure frequency. This finding is considered important since it is similar to behavioral measures of hearing losses which are commonly reported. The effect of salicylate was not frequency specific and was found at all frequencies measured. The decrements in N_1 from both intense tones and sodium salicylate were shown to be temporary.

Intense tones and salicylate showed an interaction in their effects on N_1 but not upon the a. c. cochlear potential. That is to say, that these agents, when given together, produced a greater temporary change in N_1 than either agent alone, but not more than would be expected from a simple summation.

APPROVED:

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TO

Jack A. Vernon

Ernest G. Wever

Wilhelm Wundt

and other giants on whose shoulders they stood,

and to a mother who dreamed,

Adelia Catterlin

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To Jack Vernon, Robert Brummett, Mary Meikle, Herlene Benson, Catherine Smith and the Kresge Crew, I owe a debt which can never be repaid.

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GLOSSARY

a. c. cochlear potential, the alternating current cochlear potential or, in this study where it is thought that no confusion can result, it is called the cochlear potential. This potential is thought to be produced by the hair cells in the cochlea (99) and is often called the cochlear microphonic.

dB, decibel. Decibels are an exponential or logarithmic unit of sound pressure level. These units express the magnitude of any sound relative (re:) to an arbitrary sound pressure level. Decibel as a mathematical expression is,

$$dB = 20 \log \frac{P_1}{P_2}$$

where P_1 is the sound pressure level of concern and P_2 is the reference pressure. Two reference pressures are in common use, 1 dyne/cm^2 and .0002 dyne/cm^2. It may be helpful to remember that .0002 dyne/cm^2 is 74 dB below 1 dyne/cm^2. A conversion can be made between these references according to the following formula,

$$\text{dB re: 1 dyne/cm}^2 + 74 \text{ dB} \Rightarrow \text{dB re: .0002 dyne/cm}^2$$

frequency function, the change in a potential as the frequency of the stimulus is changed. An example of the 1 μV a. c. cochlear potential frequency function is shown in Figure 3.

harmonic, is a component of a periodic wave having a frequency which is an integral multiple of the fundamental frequency. The

fundamental of a pure tone is also the first harmonic.

intensity function, the change in magnitude of a potential as the intensity of a stimulus is changed. Examples of intensity functions of the a. c. cochlear potential and N_1 are shown in Figures 4 and 7 respectively.

mg %, milligram per cent. Milligram per cent is the number of milligrams of solute in 100 ml of solvent, expressed as a per cent. Therefore, a blood level of 20 mg % is 20 mg. in 100 ml. of blood.

N_1, is the initial negative neural response of the cochlear nerve usually resulting from the abrupt onset of an acoustic stimulus. It is also called, improperly, the eighth nerve action potential.

octave, is the interval between two frequencies having a ratio of 2 to 1.

Preyer pinna reflex, movements of the pinna in response to the onset of an intense acoustic stimulus.

re:, relative.

rms, root-mean-square. In the case of sine waves, rms multiplied by 2.83 equals the peak-to-peak value. This conversion may be used for the a. c. cochlear potential in this study since it is a sine wave. However, this conversion may not be used for N_1 as it is not a sine wave.

salicylates, this term will be used to refer to commonly used salicylate analgesics and their metabolites. Commonly used salicylate analgesics include, acetylsalicylic acid, sodium acetylsalicylate, sodium salicylate, salicylic acid, choline salicylate, and methyl salicylate.

SPL, sound pressure level. This term, SPL, is commonly used in the abbreviation dB SPL where it refers to dB re: .0002 dyne/cm^2.

The Temporary Effects of Intense Sound and Sodium Salicylate on the Electrical Activity of the Cochlea

Two important causes of hearing loss are intense sound and ototoxic drugs. Decreases in auditory sensitivity, or hearing losses, are usually seen as a change in the threshold of hearing. These threshold shifts, produced by intense sound as well as aspirin (acetylsalicylic acid), are of considerable interest as the general population is frequently exposed to both of these agents.

A number of studies have pointed to the cochlea as the site where both intense sound and salicylate[1] produce their effect. The purpose of the present study was to use electrophysiological measures of cochlear function to specify those changes produced by both intense sound stimulation and salicylates. The parameters measured were the a. c. cochlear potential, which is thought to reflect activity of the receptor cells, and the auditory evoked neural potential (N_1), which reflects activity of primary cochlear neurons. Preliminary evidence has suggested that these two measures react differently. A further related question of interest was the possibility that these agents might interact when given simultaneously. In addition, the techniques that have been previously used to study the evoked neural activity from the cochlea have suffered from limitations such as inadequate frequency range and insufficient attention to sound quantification. Therefore, the present study was designed to contribute

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[1]The term salicylates will be used to refer to commonly used salicylate analgesics and their metabolites.

quantitative data to the cochlear electrophysiological literature.

It is thought that changes in cochlear function, as measured here, reflect the hearing losses that have been shown to be produced by both intense sound and salicylates. Behavioral studies showing hearing losses, as well as morphological and electrophysiological studies which have shown changes in the cochlea, have been reported for both intense sound and salicylates. A review of the literature on the effects of salicylates and intense sound will set the stage for the present study.

The Effects of Salicylates on the Auditory System

Most people who take large doses of salicylate, such as ten to fifteen aspirin tablets (5 grain) per day, experience temporary hearing threshold shifts (TTS) as a side effect. This temporary hearing deficit from salicylate stands in contrast to other ototoxic drugs, such as streptomycin, which produce permanent hearing losses.

Aspirin is a commonly ingested non-prescription drug, so common that in 1964, for example, sixteen billion aspirin tablets were sold in the United States alone (93). Some people, such as those suffering from rheumatoid arthritis, who take large doses of aspirin achieve high concentrations of salicylate in the blood. Although high concentrations are necessary for the beneficial effects in the treatment of rheumatoid arthritis and rheumatic fever, these high concentrations produce side effects of gastrointestinal bleeding, temporary hearing loss, tinnitus, nausea, vomiting and dizziness (33, 22, 44, 29, 107). The pharmacological actions of salicylates are

summarized in Appendix 1.

A number of biochemical studies have been done in attempts to elucidate the biochemical mechanisms underlying these pharmacological actions. An outline of these biochemical studies can be found in Appendix 2. Smith and Smith (86) suggest that the ototoxicity of salicylate is due to the uncoupling of oxidative phosphorylation. No data to support this hypothesis are given and therefore this remains only a suggestion.

Before reviewing the literature concerned with hearing impairment due to salicylate, something should be mentioned about salicylate metabolism. After ingestion, aspirin is rapidly hydrolyzed and metabolized into a spectrum of related compounds. These compounds are shown in Appendix 3. The clinical effects of aspirin can largely be attributed to the salicylate anion liberated in the body, by hydrolysis (107). The use of sodium salicylate, rather than acetylsalicylic acid, can provide the salicylate anion and by-pass the acetyl hydrolysis (Reaction No. 1, in Appendix 3). Therefore, in this study, sodium salicylate will be administered and the amount absorbed by the animal will be measured as the concentration of total salicylates in whole blood.

Several authors have attempted to define the relation between aspirin dosage, blood levels of salicylates, and the associated hearing losses in man (22, 33, 61, 68, 69, 96). These relationships are not tightly coupled but as the blood levels increase above about 20

mg %[2], hearing losses of 30 dB or more have been measured (61, 68). Thus, rheumatoid arthritis patients taking aspirin, who often have blood levels in the range of 20 to 35 mg %, may be expected to have hearing losses of 30 dB or greater. A point may be reached, however, at about 40 mg %, where higher blood levels do not further increase the hearing loss (69). Nevertheless, hearing losses as great as 60 dB have been reported during severe salicylate toxicity (72,96). Fortunately, the hearing loss from salicylate is reversible, usually within 24 to 72 hours (61, 69), and even patients who have taken high doses of aspirin for long periods of time (up to two years), regain their hearing when they stop taking aspirin (69).

The character of the hearing loss from salicylates is still a subject of some debate. Myers and Bernstein (69), as well as Waltner (96), have reported similar threshold shifts at all frequencies, whereas McCabe and Dey (61) report greater hearing losses at the high frequencies. Myers and Bernstein as well as McCabe and Dey used subjects with normal hearing before the salicylate ingestion, comparable audiologic methods, and they both measured salicylate blood levels. An important difference between these two studies may have been the level of blood salicylate attained. A detailed comparison cannot be made, for Myers and Bernstein report actual salicylate blood levels from only two individuals with normal hearing, whereas McCabe and Dey reported individual levels for all subjects. If the two subjects

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[2]mg % is the number of milligrams in 100 ml, expressed as a per cent. Therefore, 20 mg % is 20 mg. in 100 ml. of blood.

reported by Myers and Bernstein are representative of the blood levels achieved, these subjects did have higher blood levels (40 and 48 mg %) than the subjects in the McCabe and Dey study (a range of 22 to 35 mg % for five subjects).

Studies utilizing non-human subjects have also reported hearing losses from salicylates. Myers and Bernstein (69) studied the squirrel monkey using a conditioned avoidance technique and reported an average loss of 26 dB from a single large dose of sodium salicylate. Their doses of 500 to 600 mg/kg, given subcutaneously, produced an average blood level of 36 mg % (more than 24 hours after injection). The threshold shifts reported were the same at all frequencies tested, 250 Hz to 8000 Hz, and thus are similar to the data reported by these authors for humans.

Wilpizeski and Tanaka (105) also used a conditioned avoidance procedure on a single cat and found hearing losses of 15 to 30 dB after an intraperitoneal injection of sodium salicylate (200 mg/kg). The hearing losses at various frequencies, that they reported, were somewhere between those reported by Myers and Bernstein and those reported by McCabe and Dey. Wilpizeski and Tanaka also found hearing losses from salicylates in guinea pigs. They reported a shift of about 10 dB in the threshold of a behavioral response to a noise stimulus after an injection of sodium salicylate (300 mg/kg, injected intraperitoneally). Dederding (17) also reported hearing losses from salicylates in guinea pigs using the Preyer pinna reflex[3]

[3]Reflex movements of the pinna in response to brief acoustic stimuli.

as a measure of hearing.

In addition to the temporary hearing loss from salicylate, it should be mentioned that three cases of permanent deafness have been attributed to aspirin (25, 45, 47). These cases stand out as very unusual and may have resulted from an allergic response or some other unsuspected agent.

Neither the site nor the mechanism of action of salicylate induced hearing losses is known. In considering the site of action on the auditory system it is necessary to distinguish between peripheral and central effects. Since the hearing loss is not of the conductive type, the outer and middle ear are not implicated (69). Numerous authors have suggested the inner ear, the cochlea, as the site of action (22, 26, 38, 63, 83, 96). Myers and Bernstein (69) as well as McCabe and Dey (61) argue that higher auditory centers are not implicated as neither word discrimination scores nor threshold adaptation measures were significantly affected; however, the logic of their arguments is not entirely clear.

Theories on the cause of salicylate induced hearing loss have been sparce. Falbe-Henson (22) suggested that an increase in the intralabyrinthine pressure was responsible for the hearing losses. The similarity of the salicylate hearing loss with the loss found in Meniere's disease, which has as a major pathologic feature, evidence of increased pressure, has been cited by Waltner (96) as indirect supporting evidence of the pressure hypothesis. It should be noted that there are no reports that could be found in the literature of anyone

measuring increased pressure in the cochlea associated with salicylates.

Histological changes in the cochlea due to salicylates have been reported (22, 28, 33, 66). However, the reliability of these changes is in question. The problem of histological artifacts being reported as changes due to salicylates or other agents still exists. For example, Falbe-Henson (22) reported changes in the tectorial and Reissner's membrane. These changes were present in both drug treated and control cochleas, and both of these structures are notorious for being altered during perfusion and fixation. In general the histological changes reported in the early literature have not been replicated. In a recent study, in which both light and electron microscopy were used, no changes were found (69). Further the authors of this study question the expectation of finding morphological changes from a drug producing such a temporary loss of hearing. More recently Hawkins (38, 39) has reported occlusion of the spiral vessels within the cochlea from salicylate.

In spite of suggestions by many authors that the site of action of salicylates is intracochlear, in only three studies has the effect of salicylates upon electrophysiological measures of cochlear function been studied. Furthermore, the findings of these reports do not agree.

Electrophysiological Studies

Electrophysiological measures of the auditory system have been used in attempts to locate the site of action of salicylate (25, 63,

83, 105). Changes in both the alternating current cochlear potential[4] and evoked neural potentials have been reported in these studies.

The A. C. Cochlear Potential

There are at least four electrical potentials which can be recorded from the cochlea. Three of these potentials are direct current potentials and one is an alternating current potential. The source and function of these potentials are still a matter of some debate (90, 99).

The alternating current cochlear potential is most commonly recorded from an electrode placed on the round window membrane of the cochlea. This potential originates within the cochlea and is thought by some to be a generator potential produced by the hair cells (99). The outstanding characteristic of the alternating current potential is its reproduction of the frequency and intensity of acoustic stimuli. The range of frequency fidelity extends throughout the hearing range of all animals which have been studied. And within this range, the alternating current cochlear potential has no detectable threshold and over a large intensity range has a linear relation to the sound pressure of the acoustic stimulus. It can be measured from a level of physiological noise up to the physiological limits of the tissues of the cochlea. The linear relationship between sound pressure and the cochlear potential amplitude has been measured to be as

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[4]The alternating current cochlear potential is also called the cochlear microphonic. It will hereafter be called the a. c. cochlear potential or simply the cochlear potential.

over a range as large as 75 dB (99).

Thus the use of the a. c. cochlear potential as a measure of cochlear function is based on its intensity and frequency fidelity as well as its possible role as the generator potential which initiates activity in the cochlear nerve (99).

The Effects of Salicylate on the Cochlear Potential

Silverstein, Bernstein and Davies (83) recorded the cochlear potential from the bone near the round window of the cat. They found an average decrease of 40% in the cochlear potential after 350 mg/kg of sodium salicylate was injected intraperitoneally. McPherson (63) likewise reported a similar decrease in the cochlear potential of the guinea pig after administration of 300 mg/kg of choline salicylate, via orogastric tube. On the other hand, Wilpizeski and Tanaka (105) found no significant impairment in the ability of guinea pigs to generate the cochlear potential after they were given 300 mg/kg of sodium salicylate intraperitoneally.

The Auditory Evoked Neural Potential, or N_1

One may also record the activity of the cochlear nerve from a round window electrode. An evoked potential or N_1 measured from this vantage point represents electrical activity generated by nerve fibers in the cochlea. N_1 is thought to contain the summed activity from the unmyelinated dendritic endings, myelinated dendritic fibers, nerve cell bodies and myelinated axonal fibers. N_1 represents the number of nerve fibers activated as well as the synchrony of their

activity.

As a measure of peripheral auditory function, N_1 has several limitations. The amplitude of N_1 depends upon the rise-time of the auditory stimulus as well as its frequency and intensity (18, 80, 90). The intensity range over which N_1 can be recorded from the round window is limited due to interference from the a. c. cochlear potential. This interference arises primarily from the steep growth function of the cochlear potential intensity function. Although there are some specialized recording techniques which will allow N_1 to be recorded over a larger intensity range, because of severe procedural limitation, such methods have very restricted applicability (97).

The frequency limitations of N_1 are apparently due to the mechanics of the cochlea. Frequencies below 1 kHz produce bursts of nerve activity on every cycle of the stimulus. This is called nerve 'following'. This 'following' produces a wave form of a similar frequency as the cochlear potential, and N_1 cannot be reliably differentiated from the cochlear potential at frequencies below about 1 kHz.

The nerve activity depends upon the standing wave patterns within the cochlea. The amplitude of N_1 depends largely upon the degree of synchrony of the nervous activity within the cochlea. As the frequency of a sound is lowered the standing wave pattern apparently spreads out on the basilar membrane. When the wave pattern spreads out spatially on the basilar membrane, it also spreads out in time. Thus, the neural activity is less synchronous and hence of smaller amplitude. The processes of nerve 'following' and the concurrent

reduction in N_1 amplitude from this spreading out prevent the recording of N_1 at low frequencies (18, 90).

The Effects of Salicylate on the Auditory Evoked Neural Potentials

Four studies have reported a reduction in the auditory evoked neural potential (N_1) due to salicylates. Although the findings are in agreement, the studies are difficult to compare due to their lack of sound calibration, the use of clicks as stimuli and the manner in which they report their data. Gold and Wilpizeski (26) recorded from the cochlear nucleus region of cats and guinea pigs and found shifts of 10 to 12 dB, in the threshold of the evoked potential following a single intraperitoneal injection of 300 mg/kg of sodium salicylate. Wilpizeski and Tanaka (105) also recorded from the cochlear nucleus region in cats and guinea pigs and found a similar shift. Silverstein et al. (83) report a decrement in the evoked neural potential, recorded from bone near the round window, with an average decrease of 71% (N=5, cats). Likewise McPherson (63) found a 50 to 70% decrease in the neural potential evoked with clicks and recorded from the round window of guinea pigs.

By way of summary, it has been found that salicylates produce a temporary hearing loss, and the cochlea is implicated as a site of action. However, there are no striking morphological changes within the cochlea. Electrophysiological studies leave some doubt as to whether the cochlear potential is affected by salicylates, however, there is little doubt that the cochlear nerve potential is depressed. A purpose of the present experiment was to resolve the conflict regarding the effects of salicylate on the cochlear potential.

Additional Considerations Regarding the Salicylate Effect

Preliminary evidence, obtained in this laboratory, suggested that the effects of sodium salicylate were to increase the cochlear potential as well as decrease N_1. The effect of brainstem activation of the auditory neural efferent fibers is to increase the cochlear potential slightly as well as decrease the amplitude of N_1 (19, 20, 23, 24). The similarity between these effects suggested that efferent activation might be responsible for the sodium salicylate effect. The brainstem activation of the efferent system by salicylates was considered to be a reasonable possibility as salicylates are known to stimulate other brainstem nuclei (107). Thus the hypothesis, that the salicylate effect was due to activation of the efferent system, was made.

Another feature of the salicylate effect, noticed during preliminary studies, was its slow onset. The literature on the absorption and distribution of the salicylate anion has shown that it is dependent on the pH and the protein content of various body fluids (27, 50, 53, 56). These data suggest that the salicylate anion would enter the fluids of the cochlea slowly due to the low protein content of these fluids in comparison with plasma. Thus the hypothesis, that the slow onset of the salicylate effect is due to the slow accumulation of salicylate in the fluids of the cochlea, was made.

The Effects of Intense Sound on the Auditory System

A large body of literature has built up which describes hearing losses resulting from exposure to high intensity sounds (13, 14, 15, 54, 58, 60). The following conclusions about hearing losses from intense sound are generally accepted. 1. Stimulation with pure tones or narrow bands of noise produces a maximum threshold shift about half an octave to an octave above the stimulating frequency[5]. 2. There are no appreciable threshold shifts below the stimulating frequency. 3. These threshold shifts may be temporary and/or permanent depending on the exposure intensity and duration.

In addition to measuring behavioral threshold shifts, histological, cytological and electrophysiological changes have also been studied. The morphological and electrophysiological methods have focused primarily on the intracochlear effects of intense sound.

Histological and cytological changes in the cochlea from intense sound may be temporary or permanent (3, 4, 7, 31, 38, 39, 43, 55, 84, 88). The histological and cytological damage is thought to begin with hair cells and supporting cells and then involve the nerve endings. For example, Beagley (3, 4) reported considerable damage to hair cells and a considerable loss of the a. c. cochlear potential with no apparent damage to nerve fibers. Hawkins (38) has reported occlusion of the spiral vessels in the cochlea after noise exposure. He suggests that damage to the cochlea is in part a result of anoxia produced by

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[5]An octave is the eighth full tone above a given tone, having twice as many vibrations per second.

such occlusion.

Since the discovery of the cochlear potential by Wever and Bray (100) in 1930, attempts have been made to correlate changes in the a. c. cochlear potential with observed hearing losses (41, 42, 48, 57, 85, 101, 104). These attempts, as well as more recent efforts, have not found a good correlation (2, 3, 15, 21, 51, 73, 74, 75, 89, 95, 103).

Although a close correlation between changes in the cochlear potential and hearing losses has not been found, very intense stimulation does produce marked decrements in the cochlear potential. These cochlear potential losses usually extend across a broad range of frequencies, even though the stimulation may have been a pure tone at only one frequency. Often the cochlear potential suffered a loss at all the frequencies that were measured. These findings stand in contrast to the hearing losses which are maximum at one half to one octave above the exposure frequency.

It might be argued that Peterson's study (73) was an exception to this, in that he found maximum losses at specific frequencies. However, these specific frequency losses were found only when the overstimulation frequencies were very high, 10,000 to 40,000 Hz. Furthermore, overstimulation at 10k, 15k and 20 kHz produced maximum losses at 25 kHz in each case. In addition, when the overstimulation was at 30k and 40 kHz, the maximum loss in the cochlear potential was at the exposure frequency itself. In short, although Peterson found maximum losses at particular frequencies there was no consistent

relationship between the overstimulation frequency and the point of maximum loss.

Another frequently used electrophysiological measure of auditory function has been the auditory evoked neural potential (N_1). A number of studies have reported decrements in the amplitude of N_1, as well as in the activity of single neurons in the cochlear nerve, from intense stimulation (18, 40, 49, 59, 71, 78, 81, 87, 101, 108). However, no information about the frequency pattern of these decrements has been described.

One reason there has been a lack of information about any pattern of loss across frequencies was that, for technical reasons, clicks have been used to evoke N_1. Unfortunately, clicks contain a variety of unspecified frequencies and thus are not useful for providing differential frequency information. One purpose of the present study was to provide such information.

Hawkins and Kniazuk (40) as well as Rosenblith, Galambos and Hirsh (79) reported that at some stimulation intensities there was a reduction in N_1 without a corresponding change in the cochlear potential. This suggests that intense stimulation has its first effect, as measured electrophysiologically, on the nerve fibers. This suggestion is in contrast to the cytological findings that report the hair cell as the first structure assaulted (3, 4). Therefore, careful verification and quantification of these electrophysiological findings is important. This was another purpose of the present study.

By way of summary, it has been found that intense sound produces temporary and/or permanent hearing losses and the cochlea is a site of this action. When very intense sound stimulation is used, striking morphological and electrophysiological changes are found. These changes may also be permanent and/or temporary, reflecting the audiological findings. Although the histological evidence, by and large, suggests that the hair cells of the cochlea are the first structures damaged by intense sound, electrophysiological studies have suggested that the cochlear nerve may be the first structure affected. Thus, in this study intensities of sound which would depress N_1 without affecting the cochlear potential were of interest.

Evidence from electrophysiological studies has been presented which suggests that both salicylates and intense sound have an intracochlear site of action. A question of some clinical relevance is whether salicylates will protect the cochlea from the effects of intense sound. McCabe and Dey (61) report limited data which suggest that salicylate may exert a protective effect. Interactions between agents which damage the ear have been the subject of some research efforts (12, 16, 46, 61). These studies will be discussed later. A study of the interaction between salicylates and intense sound was another purpose of the present study.

Hypotheses of the Present Investigation

The experiments conducted in this study were designed to test five hypotheses. They are as follows;

1. The effect of sodium salicylate on the electrical activity of the cochlea is to produce a temporary reduction in the amplitude

of the evoked potential of the cochlear nerve (N_1) while leaving the a. c. cochlear potential unchanged. This hypothesis was tested in experiment 1.

2. The effect of sodium salicylate is due to an activation of the auditory neural efferent system. This hypothesis was tested in experiment 2.

3. The slow onset of the salicylate effect is due to the slow accumulation of salicylate in the fluids of the cochlea. This hypothesis was also tested in experiment 2.

4. There are certain intensities of sound which will temporarily depress N_1 while leaving the a. c. cochlear potential unchanged. This hypothesis was tested in experiment 3.

5. The effects of sodium salicylate and intense sound will interact and produce a greater temporary N_1 depression than either agent alone. Experiment 4 tested this hypothesis.

BASIC METHODS

In the series of experiments to be presented, there are certain methods used in more than one experiment. These methods will be presented before specific methods for each experiment are described.

Methods: Animal Preparation

A total of one hundred and seventeen guinea pigs weighing 280 to 850 grams (Heterogeneous Stock T strain) were used in the present investigation. Three different types of measurements were made: (1) the a. c. cochlear potential; (2) the evoked potential of the cochlear nerve (N_1); and, (3) the concentration of salicylate in the blood.

The guinea pigs were prepared for electrophysiological measurements as follows: the animals were anesthetized with allobarbital (60 mg/kg) and urethan (240 mg/kg) administered intraperitoneally. An endotracheal tube was inserted and the animals were attached to an artificial respirator. The respiration level and rate were adjusted so as to prevent any spontaneous middle ear muscle contractions (64). Body temperature, monitored by a rectal probe, was maintained between 36° and 39° C; 38.5° C. being normal. Care was taken not to overheat the animals.

Once the animal was anesthetized and respirated, one pinna was removed, usually the left, and the bulla of that ear was exposed by a postauricular incision. The bulla was then opened so as to allow visualization of the round window membrane. A silver ball electrode (about .006 inch in diameter) was placed upon the round window membrane. The electrode placement was maintained by attaching it to the bulla with

cold cure dental acrylic. The bulla remained open so that the electrode placement could be periodically checked. This opening also provided a route by which fluid accumulations could be removed when necessary. The guinea pig is notorious for its ability to generate fluids in the middle ear which in turn may "load" the ossicular chain. Care must be taken not to confuse this loading effect with that of a reduced cochlear output.

Sound stimuli from either speaker, mentioned below, were delivered to the animal through a sound cannula sealed into the external auditory meatus. The sound cannula was made in two parts (94) in such a manner that the tympanic membrane could be visualized while one part of the cannula was inserted and sealed into the external auditory meatus, thus assuring an unobstructed sound path to the tympanic membrane. Once this part of the cannula was in place the second part of the cannula, which was attached to the sound tube, was connected with a slip-fit connection. This procedure offered the advantages of protecting the ear drum while placing the sound tube, of preventing dead-ending or blocking the cannula against the meatal wall and of allowing periodic inspection of the drum during experimentation.

The side wall of the sound cannula contained a hole which allowed the insertion of a probe tube. The probe tube and its Bruel & Kjaer (B & K) condenser microphone were calibrated so that the sound intensity could be measured at the end of the sound cannula, within 5 mm of the tympanic membrane. The probe tube calibration was conducted according to the procedure presented by Vernon, Katz and Meikle (94).

Methods: Recording the A. C. Cochlear Potential

The sound stimulus utilized to produce the a. c. cochlear potential (102) was a continuous tone generated by a Western Electric 555 speaker located outside the sound shielded chamber. The equipment used to produce the continuous tones, the stimulus, is shown in Figure 1. A Bakelite tube ran from the speaker through the chamber walls to the animal, as shown in Figures 1 and 2.

The sound pressure level was measured using a 1 mm. diameter calibrated probe tube inserted through the sound cannula. The measurements of the sound pressure level utilizing this probe tube are very good. The Bruel & Kjaer condenser microphones, used with the probe tube, have an accuracy of $\pm$ 0.48 dB. Although the accuracy of specifying the sound pressure level at the ear drum is not anywhere near this great the reliability of repeated measurements of the sound pressure within 5 mm of the ear drum is good. By sealing the sound cannula into the external ear canal and specifying the frequency within $\pm$ 1 Hz, the reliability of repeated measurements of the sound intensity is within $\pm$ 1 dB.

The a. c. cochlear potential was measured using the equipment shown in Figure 2. The a. c. cochlear potential was amplified 1000 X by a Keithley Model 103 differential amplifier. The active electrode was on the round window membrane, the second active was attached to damp skin at the incision site, and the ground electrode was inserted into the ipsilateral hind foot. The animal preparation and the Keithley bioamplifier were maintained within a double walled sound

Figure 1. A diagram of the equipment used to generate a continuous tone for producing the a. c. cochlear potential.[7] FS = coherent decade frequency synthesizer, General Radio, Type 1162-A. WA = wave analyzer, General Radio, Type 1900-9001. IT = interval timer, Grason-Stadler, Model 471-1. ES = electronic switch, Grason-Stadler, Model 829-E. CT = counter timer, Monsanto Model 103-A. V = voltmeter, Simpson Model 49. Att = decade attenuator, up to 110 dB each, General Radio Type 1450-TA. PA = power amplifier, McIntosh Model 240. TE Att = tail-end attenuator, custom made with specifications to handle 100 watts continuous input. Settings of 0, 20, 40, or 60 dB available. BE = battery eliminator, Heathkit Model IP-12. Provides 7 V DC polarizing voltage for the speaker. S = speaker, Western Electric, Model 555. ⊢— An additional chassis ground. This is not always present but often needed.

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7 The frequency synthesizer served as a signal generator (accuracy ± 0.1 Hz, precision ± 1 Hz). Its output was fed into the oscillator input of the wave analyzer. The signal emerged from the tracking generator output of the wave analyzer and it was introduced into the signal input of the interval timer and the 'A' input of the electronic switch, which was in the continuous on position. The signal then went from the output of the electronic switch to the decade attenuators (220 dB). These attenuators were used to control the intensity of the signal. The output of these decade attenuators was fed into the power amplifier. The output of the power amplifier was attenuated again by the tail-end attenuator to remove amplifier noise and was then routed to the speaker.

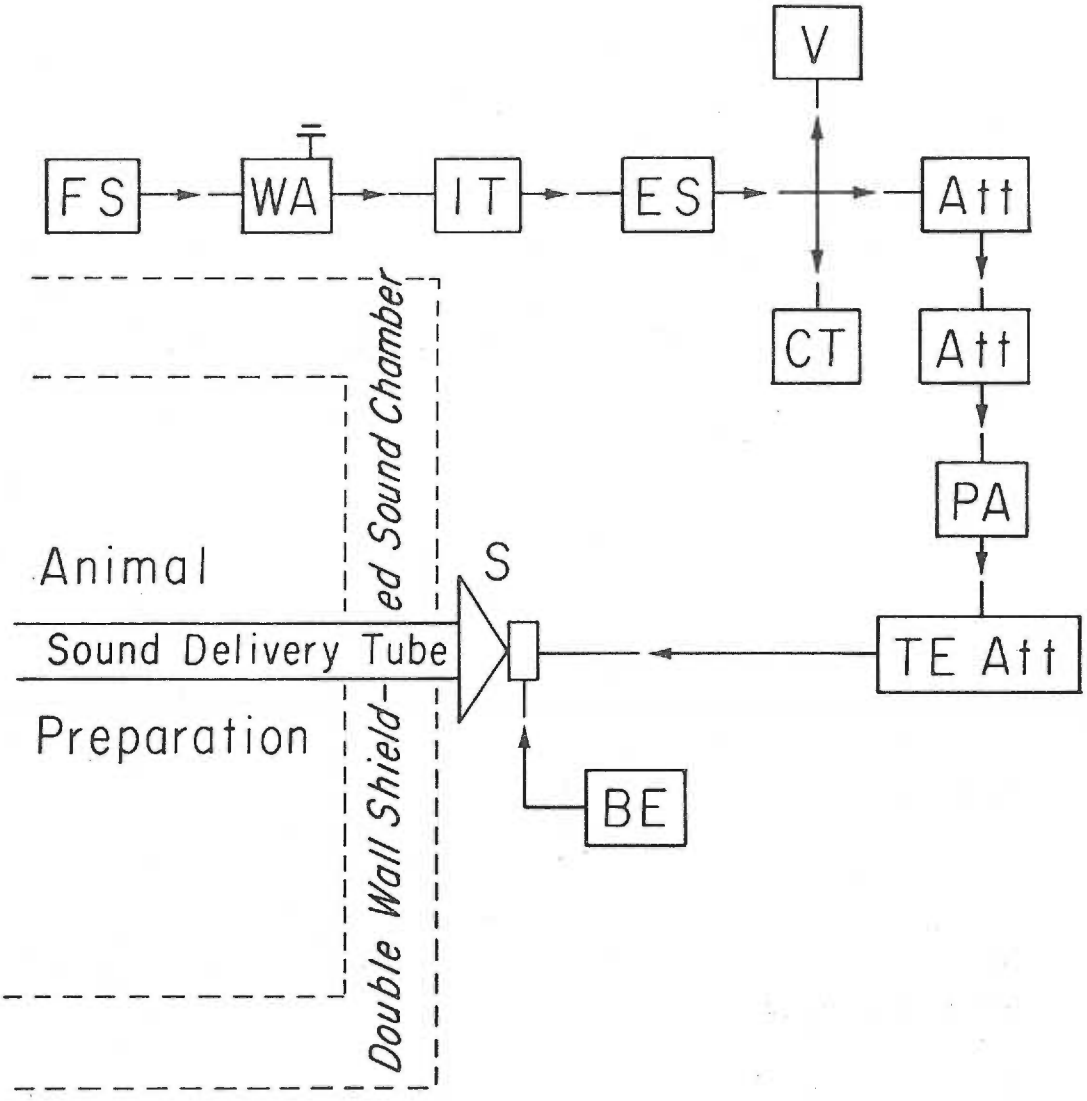

V
FS
WA
IT
ES
Att
CT
Att
PA
TE Att
S
BE
Animal
Preparation
Sound Delivery Tube
Double Wall Shielded Sound Chamber

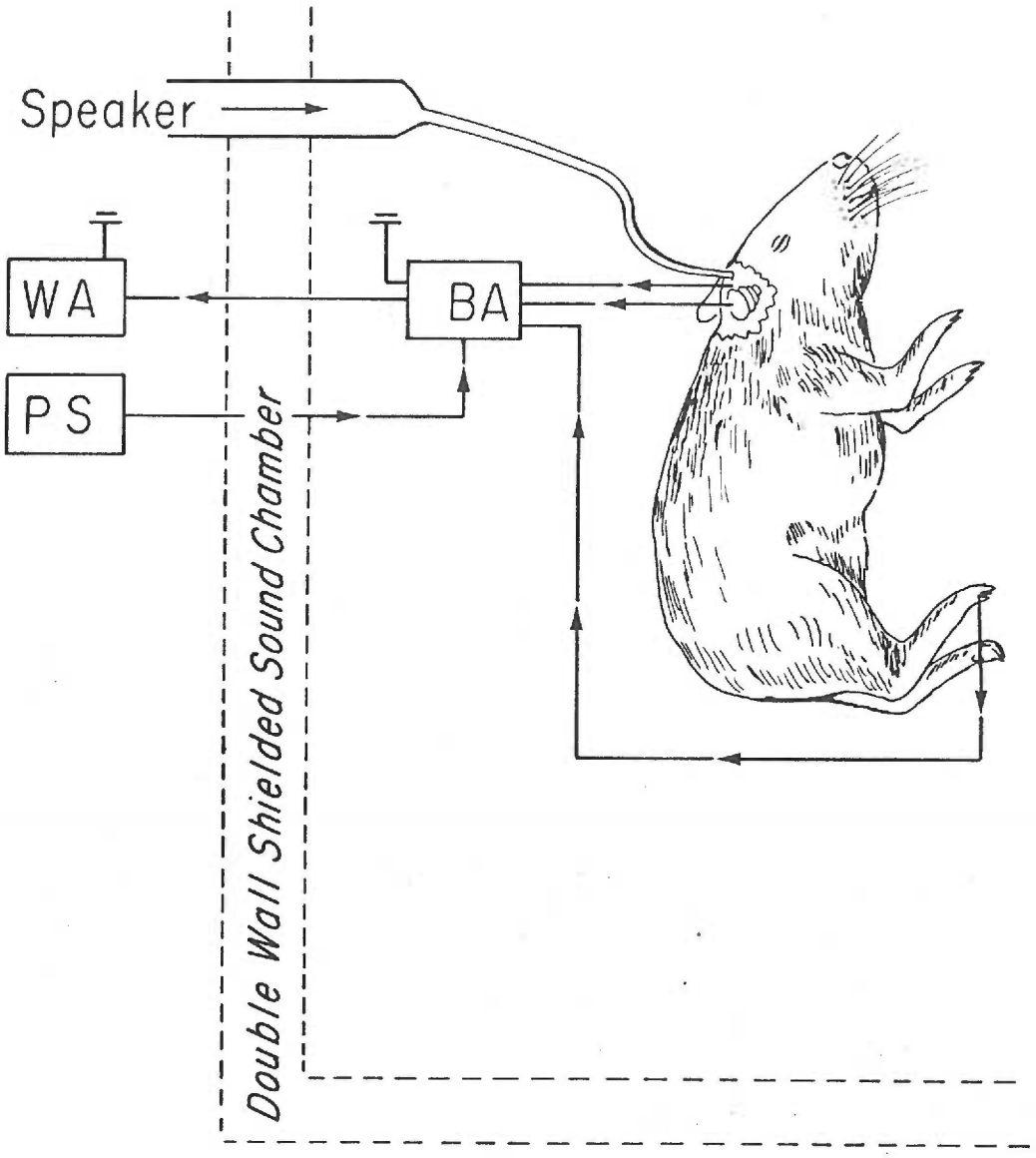
Figure 2. A diagram of the apparatus used to record the cochlear potential. The cochlear potential was read directly, as rms voltage, from the wave analyzer voltmeter.[6] BA = differential biological amplifier, Keithley Instruments, Model 103. Filter settings of 10 Hz and 100 kHz with a gain of 1000X were used. PS = power supply, Keithley Instruments, Model 1031. WA = wave analyzer, General Radio, Type 1900-9001. The 3 Hz bandwidth was used during recording. Filters at this bandwidth were such that signals ± 15 Hz were down 60 dB. SPEAKER = Western Electric 555 speaker. ⊥ An additional chasis ground. This was not always present but often needed.

6

Calibration of the biological amplifier and the wave analyzer were done at least weekly by a standard procedure. The amplification of the bioamplifier was not found to vary more than 10% from one calibration to the next. The accuracy of this calibration procedure was dependent upon the reliability (estimated to be ± 2%) of a signal read on a calibrated oscilloscope. The accuracy of this calibrated oscilloscope was in turn dependent upon the accuracy of the calibration procedure. This accuracy was stated by the manufacturer to be 3%.

The calibration of the wave analyzer was also performed approximately weekly and the variation from one calibration to the next was less than ± 1 Hz variation in frequency and less than 1 dB variation in amplitude. The accuracy of this calibration procedure is thought to be very good. The manufacturer reports accuracy of ± 2 to 5 Hz for frequency and the amplitude accuracy is ± 3% of the indicated value plus 2% of the full scale used.

Actual checks on the precision of the 1 uV cochlear potential at 1000 Hz were made with every animal. The average deviation of two observations made within 10 minutes was 0.87 dB.


Speaker
WA
BA
PS
Double Wall Shielded Sound Chamber

shielded chamber as shown in Figure 2.

The a.c. cochlear potential was measured using the equipment shown in Figure 2 and the cochlear potential was read directly from the wave analyzer voltmeter. Both the 1 uV frequency function and intensity function were recorded in this way. The 1 uV frequency function was determined by measuring intensity of sound necessary to generate one microvolt of the a.c. cochlear potential at various specific frequencies. Figure 3 shows an example of a 1 μV cochlear potential frequency function. Fourteen different frequencies between 100 Hz and 20,000 Hz were utilized, where each frequency was $\pm$ 1 Hz of the specified value. In addition, at a few specific frequencies an intensity function was determined by increasing the sound intensity, stepwise, and measuring the growth of the cochlear potential. An example of an intensity function is shown in Figure 4.

Methods: Recording the Evoked Neural Potential (N_1)

The evoked potential of the cochlear nerve (18, 80, 90) was measured using tone pulses. A different speaker than that used for the a.c. cochlear potential was used to produce the tone pulses. The speaker and other equipment used to produce the tone pulses is shown in Figure 5. Each tone pulse was 8 milliseconds in duration with a 1 msec. rise and decay time.

The equipment shown in Figure 6 was used to record the average evoked potential of the cochlear nerve (N_1). Each value for N_1 was an average from 32 stimulus presentations with 310 msec.

Figure 3. An example of a 1 μV a. c. cochlear potential from guinea pig #312-A. The amount of sound at each frequency required to produce 1 μV of cochlear potential is shown on the ordinate. For example, a level of 22 dB SPL was needed at 700 Hz, whereas at 100 Hz 49 dB SPL was required.

Actual checks on the precision of the 1 μV cochlear potential at 1000 Hz were made with every animal. The average deviation (N = 78) of two observations made within 10 minutes was 0.87 dB.

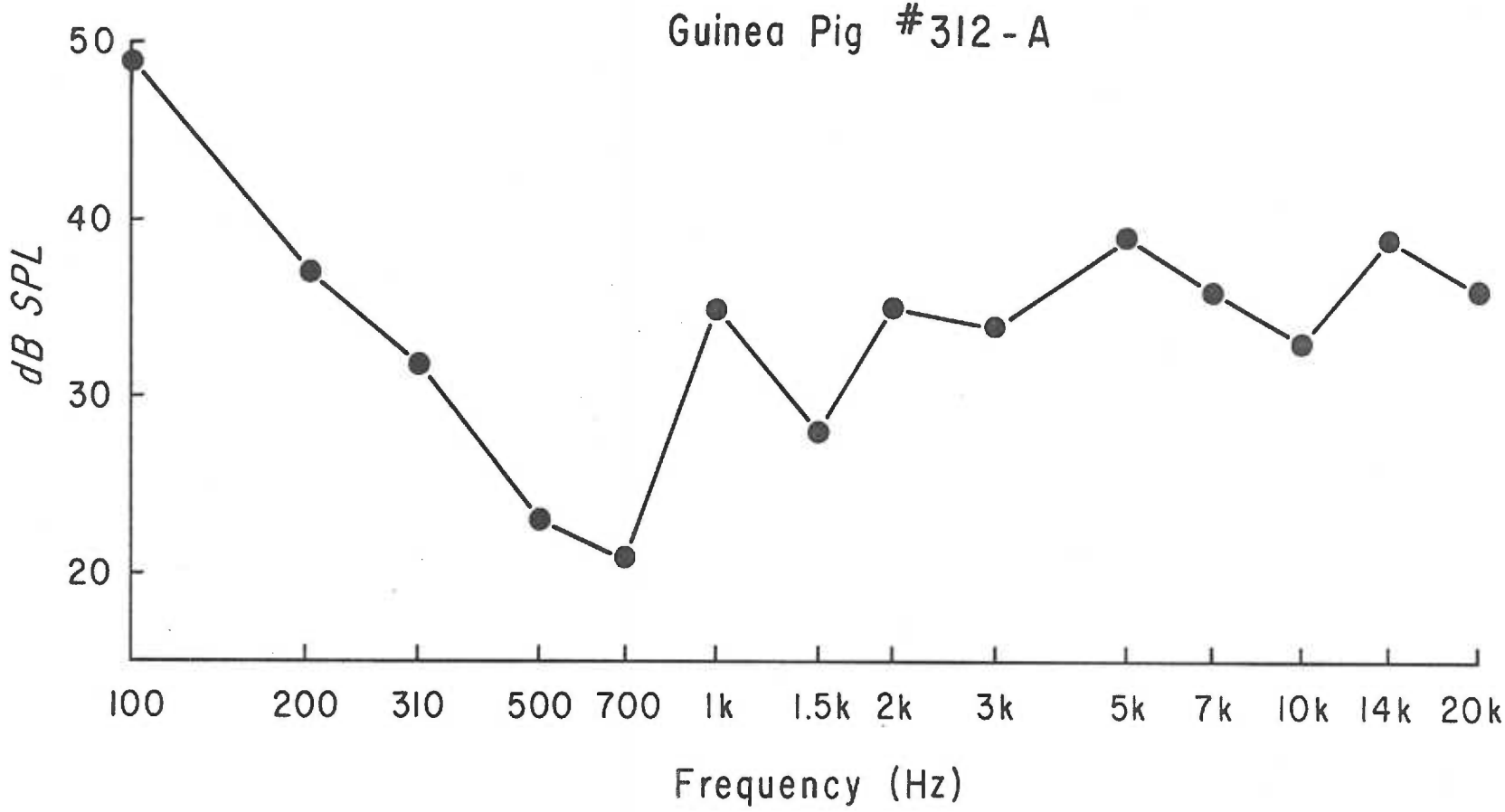

Guinea Pig #312-A
dB SPL
50
40
30
20
100
200
310
500
700
1k
1.5k
2k
3k
5k
7k
10k
14k
20k
Frequency (Hz)

Figure 4. A typical a. c. cochlear potential intensity function at 3kHz from guinea pig # 375-A. The linear portion of this function covers a considerable range. The maximum (MAX) as well as points 5 dB and 30 dB below this maximum are indicated and are discussed later in the text. Note: MAX - 30 is at 90 dB SPL and MAX - 5 is at 115 dB SPL.

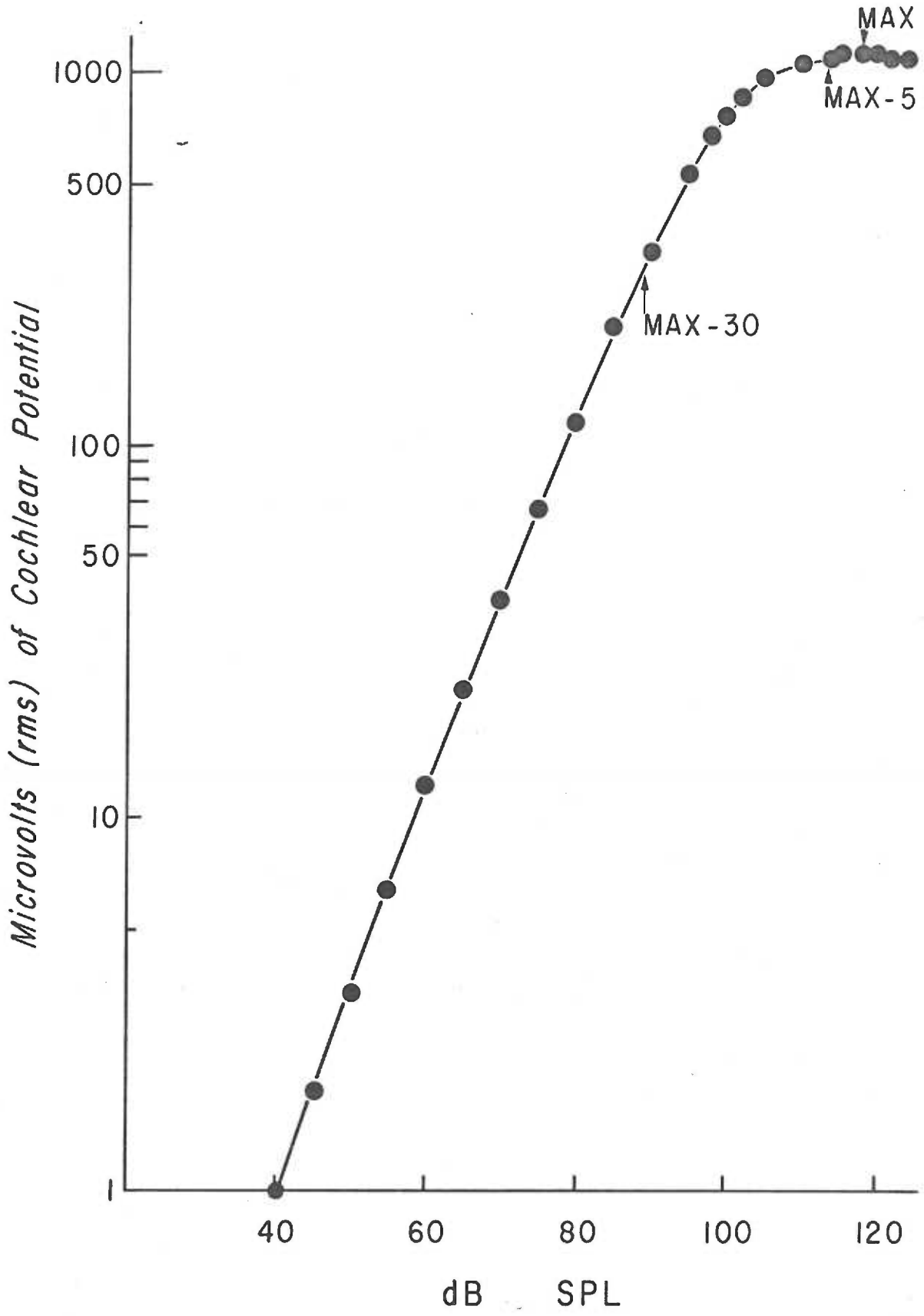

MAX
MAX-5
MAX-30
1000
500
100
50
10
1
40
60
80
100
120
dB SPL
Microvolts (rms) of Cochlear Potential

Figure 5. A diagram of the equipment used to generate the tone pulses for evoking N_1[8]. FS = frequency synthetizer, coherent decade, General Radio, Type 1162-A. WA = wave analyzer, General Radio, Type 1900-9001. IT = interval timer, Grason-Stadler, Model 471-1. PG = pulse generator, Tektronix, Type 161. WG = waveform generator, Tektronix, Type 160A. ES = electronic switch, Grason-Stadler, Model 829-E. CT = counter timer, Monsanto Model 103-A. V = voltmeter, Simpson Model 49. Att = decade attenuator, up to 110 dB each, General Radio Type 1450-TA. PA = power amplifier, McIntosh Model 240. TE Att = tail-end attenuator, custom made with specifications to handle 100 watts continuous input. Settings of 0, 20, 40, or 60 dB available. MPS = microphone power supply, Bruel & Kjaer, Type 2801. "S" = 1/2 inch B & K condenser microphone. IOWA = 10 watt amplifier, Krohn-Hite, Model DC A-104. BS = speaker, described by Beavers, Palin & Simmons (5). The configuration IOWA-BS was used as described on page 50, while the configuration MPS-"S" shown above was used as described on page 43.

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8 The signal generation and route was exactly the same as described in Figure 3. However, the signal was not a continuous tone but rather a tone pulse. The occurrence of a tone pulse was controlled by the output of the waveform and pulse generators. Their output triggered the interval timer, electronic switch, oscilloscope and Biomac (see Figure 6). The electronic switch determined the rise-decay and duration of the tone pulses.

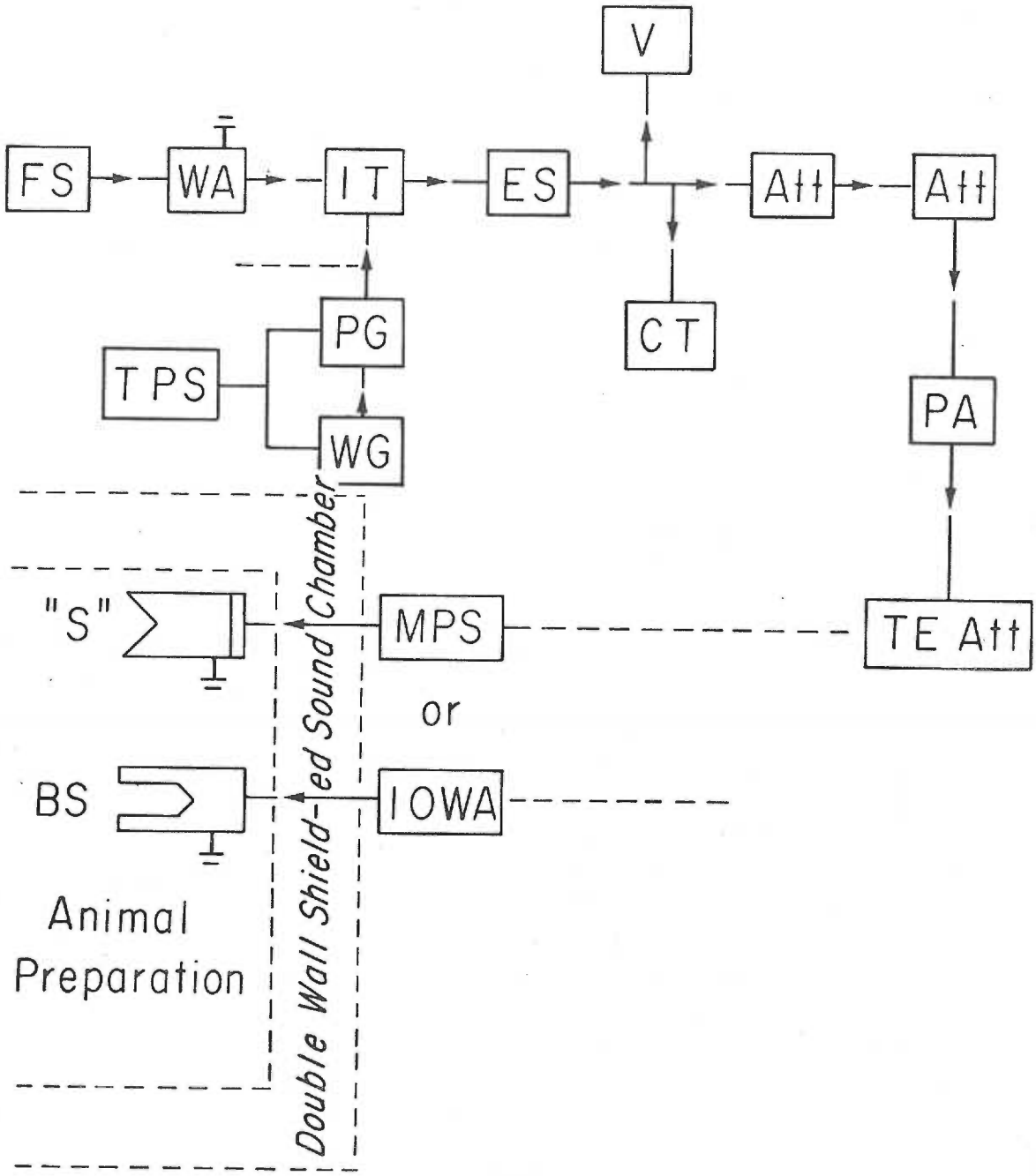
V
FS
WA
IT
ES
Att
Att
CT
PG
TPS
WG
PA
"S"
MPS
TE Att
or
BS
IOWA
Animal
Preparation
Double Wall Shielded Sound Chamber

Figure 6. The apparatus employed to record N_1[9]. OSC = oscilloscope, Tektronix, Type 564 B storage oscilloscope with plug in unit Type 349, amplifier, filters set on 0.1 Hz and 0.1 MHz. BIOMAC = BIOMAC 1000, Data labs., London. Sweep time usually 10 msec. VAR= variance unit, Type 1005, Data Labs., London. BA = biological amplifier, Keithley Instruments, Model 103. Filter settings of 10 Hz and 100 kHz. PS = power supply, Keithley Instruments, Model 1031. MPS - 'S' = microphone power supply, Bruel & Kjaer, Type 2801 and 1/2 inch B & K condenser microphone. IOWA - BS = 10 watt amplifier, Krohn-Hite, Model DC A-104, with speaker described by Beavers et al. (5). The MPS-'S' and IOWA - BS were used in different experiments as described in the Methods section.

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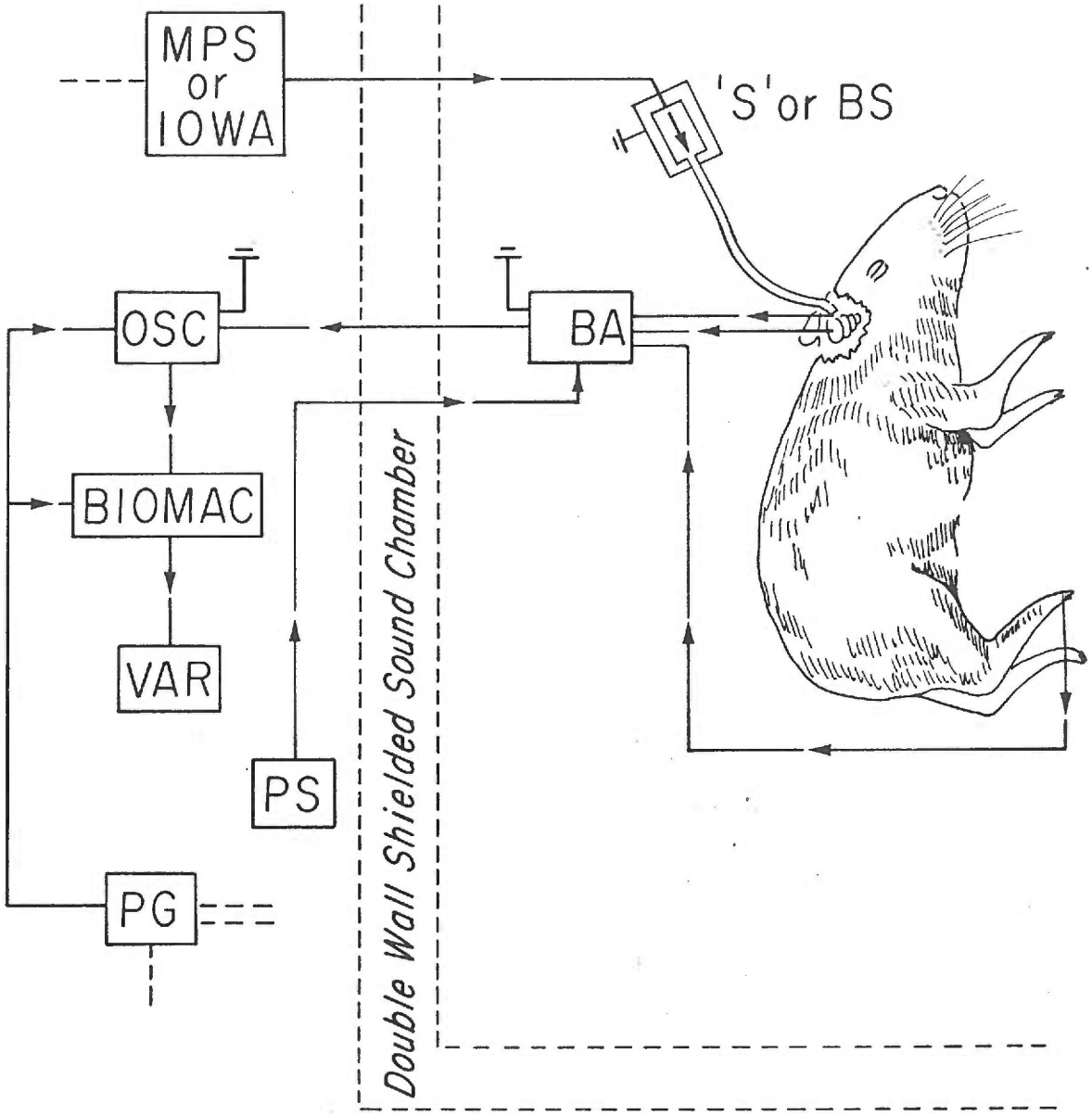
9 The bioamplifier (BA) was calibrated in the usual way. The oscilloscope was calibrated using the internal calibrator. The manufacturer's reported accuracy is 1-1/2 %. However, the oscilloscope face could only be reliably read to within about 2.5%.

Once the oscilloscope was calibrated a signal out of the oscilloscope was used to calibrate the signal averager (BIOMAC). The BIOMAC 1000 was within 5% from one calibration to the next.


MPS
or
IOWA
'S' or BS
OSC
BA
BIOMAC
VAR
PS
PG
Double Wall Shielded Sound Chamber

between them (See Appendix 4 for a justification of the number of sweeps per average and the stimulus repetition rate). The onset of the tone pulses was random with respect to phase in order to cancel the cochlear potential from the N_1 average. The N_1 response was averaged with the BIOMAC 1000. A peak-to-peak (p-p) measure of N_1 amplitude (97) was read from the BIOMAC visual display after centering it on a grid (See Appendix 4).

An intensity function of N_1 was determined at each of the frequencies of concern. In general, the intensity functions were simply the growth of N_1 magnitude with increasing signal intensity and ran from about 20 μV to 150 μV. Examples of N_1 intensity functions are shown in Figure 7. Changes in the intensity function were measured in dB on the horizontal axis and will be discussed in more detail later. As with the cochlear potential, electrical radiation artifacts were eliminated.

Methods: Determining Blood Concentrations of Salicylates

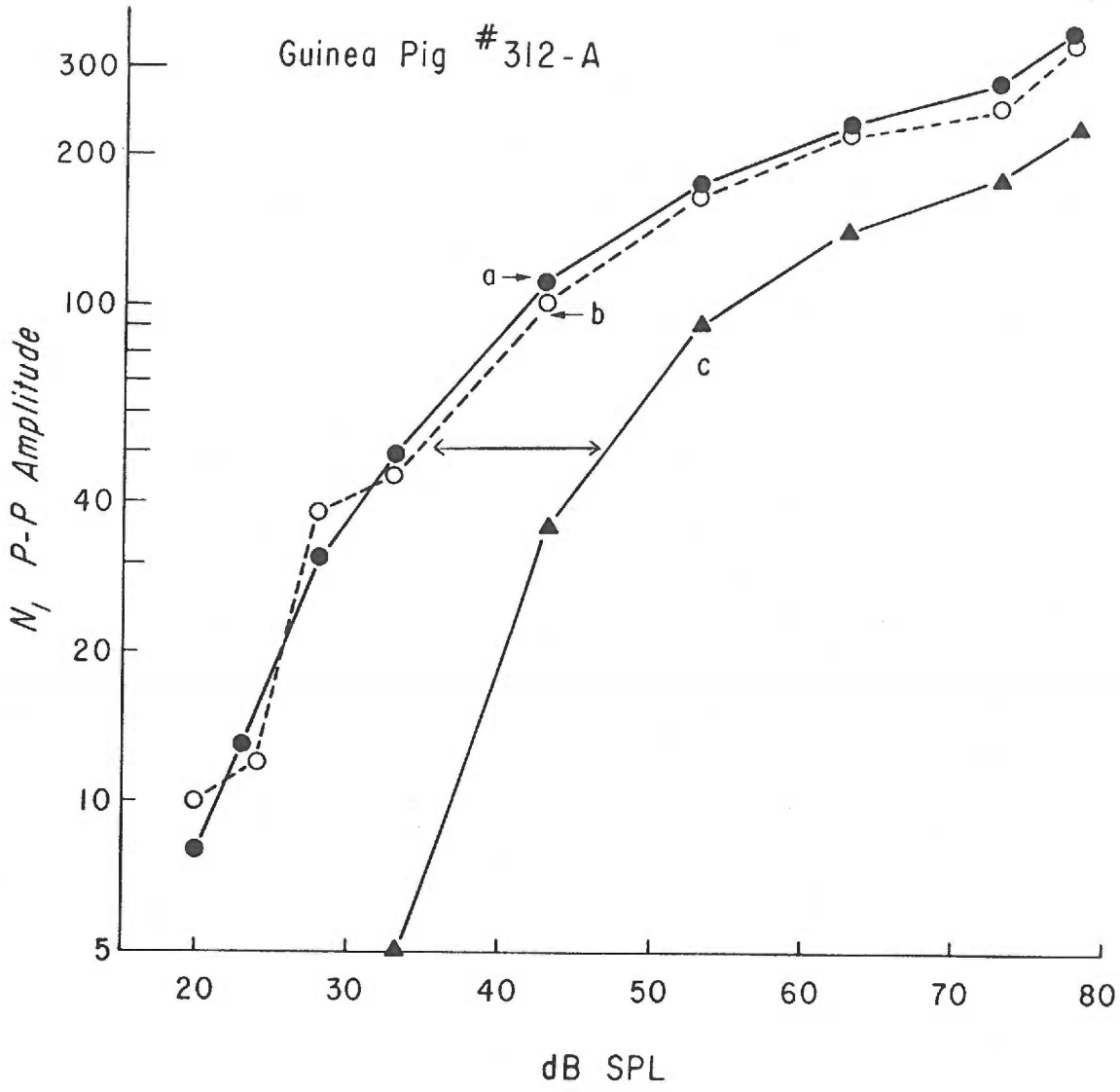
All injections of sodium salicylate or placebo were single subcutaneous injections given on the animal's back. Three different dosages of sodium salicylate were used: 286 mg/kg, 500 mg/kg and 545 mg/kg (See Appendix 3). The animals given a placebo were injected with isotonic saline in an equivalent volume (5 ml/kg).

Blood samples were usually taken from a jugular vein catheter at specific intervals after the injection of sodium salicylate or isotonic saline. Each sample was 0.2 milliliter (ml) in volume

Figure 7. Intensity functions of N_1 elicited by a 5919 Hz tone pulse from guinea pig #312-A.

Curves 'a' and 'b' The means of the peak-to-peak amplitudes are shown from two recording times 2-1/2 hours apart. Curve 'a' was recorded at 11:40 am and curve 'b' was recorded at 2:10 pm. Repeated N_1 amplitudes, in the range of 30 to 70 μV, measured within a half hour had an average deviation (N = 40) of 13 %.

Curve 'c' These data were recorded at 5:49 pm and show a shift in N_1 due to salicylate. The change in the amount of sound, i. e. the dB distance on the horizontal axis, necessary to produce 50 μV of N_1 is used as a measure of the change in the intensity function. This will be discussed further in the Results section.


Guinea Pig #312-A
300
200
100
40
20
10
5
N_1 P-P Amplitude
a
b
c
20
30
40
50
60
70
80
dB SPL

and was analyzed for total salicylates in whole blood by Trinder's method (92).

The method of Trinder is a colorimetric method based on a reagent containing ferric nitrate, mercuric chloride and hydrochloric acid. This reagent precipitates red blood cells, platelets and proteins in the blood and reacts with salicylates to give a purple color. The method is reported to have low blank values for whole blood (equivalent to 1.2 - 1.8 mg%) and good recovery of salicylate (99.4 - 100.5%) (92).

The method was modified for small samples and the following procedure was used.

1. 0.2 ml of whole blood or an appropriate standard solution was delivered into a testube with a 1 cc disposable syringe. Three ml of the color reagent was then pipeted into the test tube.

2. The resulting precipitate of denatured proteins was mashed against the bottom and sides of the test tube until it was finely dispersed. Incomplete dispersion of this precipitate was found to produce low salicylate values and therefore considerable care was used to insure complete break up of the precipitate.

3. The samples were then centrifuged for 5 minutes at 2450 X g. in an International Equipment Co. Model HN centrifuge.

4. The supernatant liquid was decanted into the colorimeter tubes and the percent transmittance was read at 540 mu in a Spectronic 20 Spectrophotometer (Bausch and Lomb).

5. The transmittance was then converted to absorbance using a

chart. Standard curves of absorbance versus mg% of salicylic acid were plotted. An example of two standard curves obtained more than a year apart are shown in Figure 8. A standard curve was routinely plotted with each analysis and was visually compared to previously obtained standard curves. The absolute values of the standards as well as the slope of the standard curve were not found to vary appreciably.

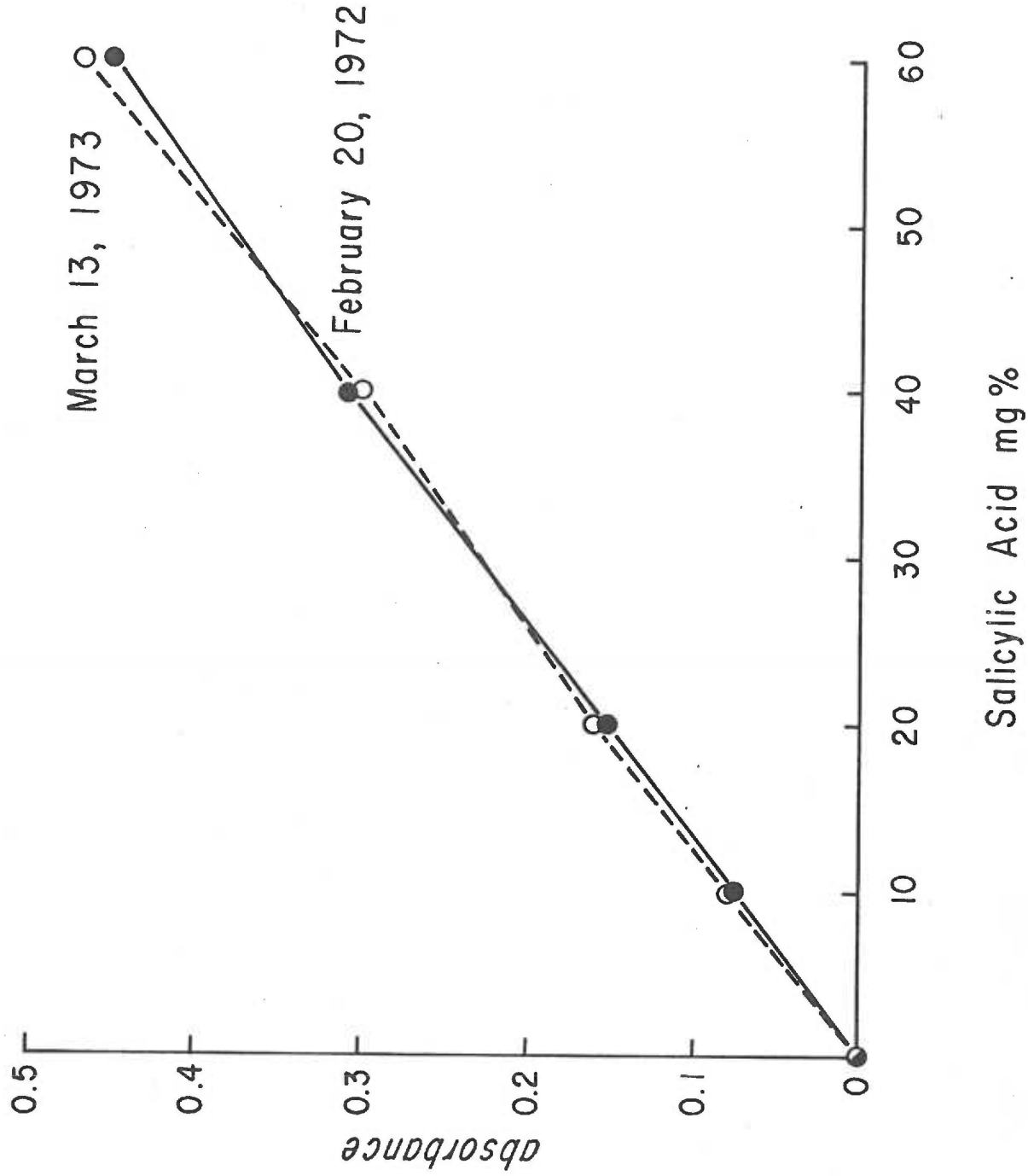
6. The transmittance values of blood samples were also converted to absorbance using the chart values and a comparison with the standard curve gave a value of the mg%.

The blank values for guinea pig whole blood were not greater than 2 mg% when compared with standards prepared with distilled water. These values compare well with Trinder's blank values of 1.2 to 1.8 mg% found with human blood. Neither the anesthetics used nor sodium heparin raised the blank values.

In general the reliability of the method was good and analyses of duplicate samples were usually within 2 mg %. The techniques used for blood collection and storage did not affect the amount of salicylate found. This method has the limitation of not reacting with all the metabolites of sodium salicylate. In particular the ether glucuronide does not react to give a color. (See Appendix 3, the ether glucoronide is shown as the result of Reaction No. 3 in Figure 30.) Although the exact amounts of this metabolite in the blood of guinea pigs is not known, the presence of glucoronides in the blood of man and the rat are reported to be so low as to be insignificant or

Figure 8. Standard curves of absorbance at 540 mμ plotted versus mg % salicylic acid.

These curves are typical of those done with each analysis.


March 13, 1973
February 20, 1972
absorbance
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
50
60
Salicylic Acid mg %

barely detectable (70) due to their rapid removal by the kidney.

Methods: Overstimulation Tones

Pure tones of 3 kHz, 6 kHz and 12 kHz were used for overstimulation of the guinea pigs ears. In order to check the purity of the tone which was used for intense stimulation it was analyzed with the wave analyzer. While the tone was on continuously, both harmonic distortion and energy at one-half octave above the fundamental were measured. No appreciable sound pressure was found one-half octave above the fundamental. In the worst case, at 3 kHz, the second harmonic was 25 dB below the fundamental. The frequency analysis of the 3 kHz, 6 kHz and 12 kHz overstimulation tones are shown in Appendix 7.

The a.c. cochlear potential intensity function was used as a guide to determine an exposure intensity that was "tailor made" to each animals ear (74, 75). Once the particular frequency, e.g. 3 kHz, was picked for exposure an intensity function, the maximum at that frequency, was determined as follows. The intensity function was measured using short duration tones, about 3 sec, presented in increasing intensities. Initially the intensity was increased in 5 dB steps and as maximum was approached 2 dB steps were used. The highest cochlear potential reading in the series was accepted as the maximum. If two sound pressure levels produced identical readings of the cochlear potential, the maximum (MAX) was calculated as midway between them.

Once maximum was determined the sound exposure intensity

could be set. In this experiment sound exposures were made 5 dB, 30 dB or 55 dB below maximum. These sound exposure intensities are referred to as MAX -5, MAX -30, etc. They are shown in Figure 4.

SPECIFIC METHODS

The present investigation consisted of four experiments, each of which had method variations from the Basic Methods. Each experiment also had different procedures as well. Therefore, a procedural summary and method variations for each experiment will be described.

Experiment 1 Method: Determining Salicylate Effect

This experiment was divided into two parts: a short term part (6 hours post injection) and a long term part (5-168 hours). In both parts the study was conducted and the data initially analyzed using a single blind procedure.

Short Term Part A summary of the procedure used in this part was as follows:

1. The guinea pigs were anesthetized, an endotracheal tube and an intravenous catheter were inserted. A blood sample was then taken. The bulla was opened and the electrode placed on the round window membrane.

2. The 1 μV frequency function of the a.c. cochlear potential was recorded.

3. The intensity functions of N_1 were recorded, at the middle and high frequencies.

4. A single injection of sodium salicylate or saline was administered subcutaneously.

5. A blood sample was taken half an hour after injection and then at hourly intervals for six hours.

6. The intensity functions of N_1 were recorded at hourly intervals for six hours. The a.c. cochlear potential 1 uV frequency function was recorded 3 and 6 hours after injection.

7. The sound systems were calibrated and the animal was sacrificed with an overdose of anesthetic.

The surgical preparation and the cochlear potential recordings were the same as described in the Basic Methods. The evoked neural potential was recorded at middle frequencies (4283-5900) and high frequencies (10008-11120 Hz). Three groups of six animals each were used in this experiment. Dosages of 286 mg/kg and 545 mg/kg of sodium salicylate were given to two groups and the third served as a control. One animal died in the 286 mg/kg group and two died in the 545 mg/kg group.

The tone pulses used to evoke N_1 is experiment 1 were produced by driving a Bruel & Kjaer one-half inch condenser microphone in reverse as a "speaker." This "speaker" produced tone pulses which contained a minimum of on-set and off-set transients. Unfortunately this speaker was very sensitive to alterations in acoustical impedance and resonance properties of the sound delivery system. Thus, the frequencies at which the tone pulses contained minimal on-set transients varied from one sound field to the next,

or from one animal to the next. In an attempt to maintain tone pulses with minimal on-set transients, slightly different frequencies were used. These variations in frequency were not great, so that the frequency of the tone pulse signals varied only slightly from one animal to the next.

It turned out that the intensity of the tone pulses from this speaker could not be measured with the calibrated probe tube as the speaker did not produce sufficient intensities to activate the probe tube. Therefore, a substitution technique was employed to measure the tone pulse intensities. This method was merely to substitute a quarter-inch Bruel & Kjaer (B & K) calibrated microphone at the end of the sound cannula where the animal had been previously located. The errors inherent in this substitution method are probably neither much greater nor much different from those inherent in the probe tube method (94).

Long Term Part A summary of the procedure used in this part of experiment 1 was as follows:

1. The guinea pigs were injected with sodium salicylate or saline subcutaneously.

2. At a specified time, five, eight, twenty-four or 168 hours later, the guinea pigs were anesthetized and an endotracheal tube was placed. The bulla was opened and the electrode placed on the round window membrane.

3. The 1 μV frequency function of the a.c. cochlear potential and an intensity function of N_1 were recorded.

4. The guinea pig was decapitated and a blood sample taken.

The surgical preparation described in the Basic Methods was modified slightly, in that the electrode was not secured to the bulla with dental acrylic. Otherwise, this procedure as well as the method for recording the cochlear potential were the same as described in the Basic Methods section. N_1 was evoked using a 10 kHz tone pulse.

This long term part of experiment 1 used eight animals as controls and fourteen experimental animals which were injected with sodium salicylate (500 mg/kg). This part was also conducted and the data initially analyzed in a single blind procedure.

Experiment 2 Methods: Cochlear Perfusion

Twenty-three guinea pigs were used in this experiment. The surgical preparation of the animals was similar to that described in the Basic Methods section except for the following modifications. The ventral portion of the bulla was exposed first. This ventral exposure required breaking away part of the jaw and the styloid process. After the cochlea was exposed via this ventral approach, a postauricular opening was made in the bulla.

When the cochlea was exposed both ventrally and postauricularly, the electrode was placed and secured on the round window membrane as described in Basic Methods. Then, using the ventral bulla opening for visualization, a hole (.012 inch) was drilled into the scala vestibuli in the basal turn of the cochlea. A pipet filled with synthetic perilymph (as described in Appendix 5) was then placed into the scala vestibuli using a micromanipulator.

A fluid-tight seal of the pipet was obtained by using a liquid adhesive as a gasket. The pipet, once in place, was secured with cold cure dental acrylic. Stabilization of the pipet was imperative to avoid leaks or breaking the embedded tip. The second pipet was placed in the scala tympani utilizing the post auricular opening in the bulla. This pipet was also stabilized with dental acrylic. A diagram of the arrangement of these pipets is shown in Figure 9.

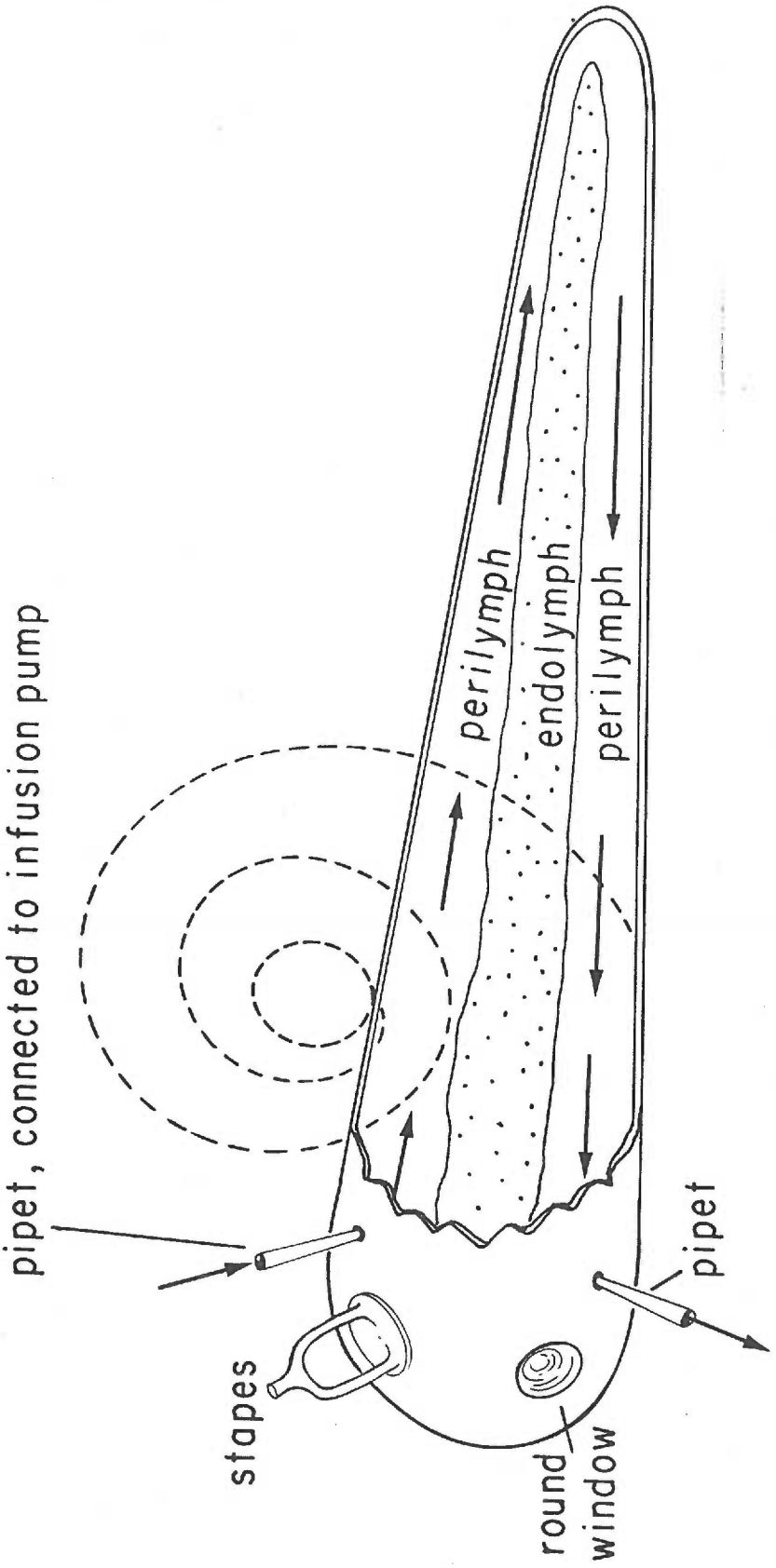
The pipet in the scala vestibuli was attached to a syringe by a flexible polyethylene tube. The syringe, during perfusion, was driven at a constant speed by a mechanical infusion/withdrawal pump. The pipet in the scala tympani served as the exit for the perfusate.

All perfusions were made at the rate of 0.1 ml/min. for 2 minutes, for a total perfusion volume of 200 ul. Based on measurements in this laboratory the volume of the perilymphatic fluid of the cochlea is about 20 ul, therefore a 200 ul perfusion was thought to be sufficient to completely replace the natural perilymph with the synthetic perilymph. In this experiment the synthetic perilymph described by Brummett, Himes and Mitchell (9) was used. The composition is included in Appendix 5.

The procedure for recording the a. c. cochlear potential and N_1 were the same as described in the Basic Methods section. The recordings were made at the following times:

1. After postauricular and ventral exposure of the cochlea and placement of the round window electrode.

Figure 9. A schematic diagram of the cochlea showing placement of the pipets used in perfusing the perilymphatic spaces of the cochlea. The cochlea is shown uncoiled in this diagram.


pipet, connected to infusion pump
perilymph
endolymph
perilymph
stapes
round window
pipet

2. After placing the first pipet in the scala vestibuli.

3. After placing the second pipet in the scala tympani.

4. After each perfusion of the cochlea.

Experiment 3 Methods: Intense Stimulation

A total of thirty-two guinea pigs were used in this experiment, however two died and their data are not reported. This experiment contained a short and a long term part. The short term part of the experiment demonstrated the effects of intense sound and used eight control and twelve experimental animals. The long term part of the experiment showed that the effect was temporary and used four control and eight experimental animals. The procedure for each of these parts is outlined below.

Short Term Part

1. The guinea pig was anesthetized and the electrodes placed as described in the Basic Methods section.

2. The 1 μV cochlear potential was recorded, at fourteen frequencies, over the range from 100 Hz to 24 kHz.

3. Cochlear potential intensity functions were recorded at the exposure frequency and a half octave above it.

4. N_1 was recorded at eight frequencies, over the range from 2 kHz to 24 kHz. An intensity function at each frequency was recorded that extended over the response range from below 25 μV to over 100 μV.

5. The ear was exposed to a pure tone at 3 kHz or 6 kHz for 15 minutes or to 12 kHz for 30 minutes, during which time the cochlear potential was continuously recorded and any middle ear

muscle activity was noted. Very little middle ear muscle activity, spontaneous or evoked, was ever seen.

6. Immediately after exposure, the intensity functions of N_1 were again recorded.

7. The 1 μV cochlear potential frequency function was recorded again, as in 2 above.

8. The cochlear potential intensity functions were recorded again, as in 3 above.

9. Both of the sound systems, for pulses and continuous tones, were calibrated using a calibrated probe tube and the animal was sacrificed.

Detailed methods for the surgical exposure of the cochlea were the same as described in the Basic Methods section.

The a.c. cochlear potential was recorded as described in the Basic Methods section, except that a few different frequencies were used. The 1 uV frequency function was recorded at the following frequencies: 100 Hz, 200 Hz, 310 Hz, 500 Hz, 700 Hz, 1 kHz, 2 kHz, 3 kHz, 4242 Hz, 6 kHz, 8484 Hz, 12 kHz, 16968 Hz, and 24 kHz.

Tone Pulses were used to evoke N_1 at eight frequencies 2 kHz, 3 kHz, 4242 Hz, 6 kHz, 8484 Hz, 12 kHz, 16968 Hz, and 24 kHz. A new speaker, described by Beavers, Palin and Simmons (5) was used to produce the tone pulses. This speaker allowed the production of pure tone pulses at frequencies over the entire range of 2 kHz to 24 kHz with a minimum of onset transients. Since the frequency characteristics of the tone pulses used in this experiment were of

extreme importance, they deserve further mention.

The tone pulse frequency characteristics were verified by two different methods. Trains of tone pulses, 3 msec. apart, were delivered to the Beavers et al. speaker (5) and the acoustic output was monitored by using a quarter-inch B & K calibrated microphone. Visual comparisons of the electrical signal delivered to the speaker and its acoustic out put, as monitored by the calibrated microphone, were made on a storage oscilloscope. Very little distortion was seen in the acoustic signal, at any frequency, using this method. However, this visual comparison method will detect distortion only when it is greater than about 5 %. Therefore, the out-put of the calibrated microphone was monitored on the wave analyzer and the energy present at frequencies between 100 Hz and 40 kHz were measured. The energy present was measured in 100 Hz steps (50 Hz bandwidth) over the range of 100 Hz to 20 kHz and in 1000 Hz steps between 20 kHz and 40 kHz. These data are presented in Appendix 6.

Long Term Part The animals used in the short term part of this experiment did not show complete recovery of N_1 during the course of the experiment, which was about 5 hours. It was thought that deterioration of the preparation and anesthesia prevented recovery of N_1. Therefore, in the long term part of this experiment, animals were exposed to the appropriate stimulus and at a specific time later they were evaluated electrophysiologically. This procedure is referred to as the long term part of this experiment

and an outline of the procedure is as follows:

1. The guinea pigs were anesthetized with sodium pentobarbital (35 mg/kg) and the left ear was exposed to 6 kHz for 15 minutes. Anesthesia eliminated middle ear muscle contractions and allowed accurate calibration of the intensity of the exposure tone with the probe tube.

2. The guinea pigs were then allowed to recover 8 or 23 hours and then they were reanesthetized with allobarbital and urethan, the electrodes placed, and recordings of the 1 μV cochlear potential frequency function and N_1 intensity functions were made.

3. Both of the sound systems were calibrated with the probe tube using continuous tones, after which the animal was sacrificed.

The anesthesia and surgical exposure of the cochlea were the same as described in Basic Methods. An exception to this procedure was made in this part of the experiment. In these animals the first anesthesia was accomplished with sodium pentobarbital (35 mg/kg) injected intraperitoneally. In the long term part it was not possible to directly measure MAX -5 and MAX -30 before exposure, as the electrodes were not placed until after exposure. Therefore, MAX was calculated from the measured values in the short term part of this experiment. At 6 kHz, MAX -5 was calculated to be 104 dB SPL and MAX -30 was 79 dB SPL.

Experiment 4 Methods: Interaction

A total of twenty-two guinea pigs were used in this experiment, however three died. This experiment contained a short term

part demonstrating the interaction and a long term part showing that the effect of the interaction was temporary. In each part, there were three groups of animals. The groups were as follows: 1) Salicylate and Overstimulation. Sodium salicylate (500 mg/kg) was injected subcutaneously and four hours later the ear was exposed to a 6 kHz tone at MAX -5 for 15 minutes. 2) Saline and Overstimulation. Isotonic saline (5 ml/kg) was injected and four hours post injection the ear was exposed to intense sound stimulation, 6 kHz at MAX -5 for 15 minutes. 3) Salicylate and Moderate Stimulation. Sodium salicylate (500 mg/kg) was injected subcutaneously and four hours later the ear was exposed to moderate stimulation, 6 kHz at MAX -30 for 15 minutes.

Short Term Part The sequence of events in this part was as follows:

1. The round window electrode was placed in the usual manner and the 1 μV cochlear potential frequency function was recorded.

2. The cochlear potential intensity function was recorded at 6 kHz using 5 and 2 dB steps to locate the maximum.

3. The intensity functions of N_1 were recorded.

4. A control blood sample was taken from the jugular vein.

5. An injection of sodium salicylate (500 mg/kg) or saline (5 ml per kg) was administered subcutaneously.

6. A second blood sample was taken about four hours after the injection.

7. The 1 μV cochlear potential frequency function was then recorded again.

8. The intensity functions of N_1 were again recorded.

9. The ear was exposed to a 6 kHz tone at MAX -5 or MAX -30 for 15 minutes.

10. The intensity functions of N_1 and the 1 μV cochlear potential frequency function was recorded again.

11. A third blood sample was taken immediately after the recordings were made.

12. The sound systems were calibrated in the usual manner, and the animal was sacrificed.

The anesthesia and surgical exposure of the cochlea was the same as described in the Basic Methods section. In addition a jugular vein catheter was placed. The recording of the a. c. cochlear potential and N_1 were accomplished as described in the Basic Methods section. The tone pulses used to evoke N_1 were produced by the Beavers et al. speaker as described in experiment 3.

Long Term Part The procedure for the long term part was as follows:

1. The guinea pigs were injected with sodium salicylate or saline.

2. About three and a half hours after injection they were anesthetized with sodium pentobarbital (38 mg/kg).

3. At four hours after salicylate or saline injection, the left ear was exposed to 6 kHz at MAX -5 or MAX -30 for 15 min. After exposure they were returned to their home cage and allowed to recover.

4. Forty-eight hours after injection the animals were

reanesthetized with allobarbital and urethan, and the electrodes were placed in the usual manner.

5. The 1 μV cochlear potential frequency function was recorded.

6. The cochlear potential intensity function was recorded at 6 kHz, using 5 and 2 dB steps.

7. The intensity functions of N_1 were recorded.

8. The sound systems were calibrated utilizing the probe tube.

9. A blood sample was taken and the exposed cochlea was perfused for histological examination.

The methods used in this part were the same as in the short term part except that calculated values of MAX -5 and MAX -30 were used to determine the stimulating intensity as the recording electrodes were not placed until after exposure. All available MAX values for 6 kHz were used to calculate the values of MAX -5 and MAX -30.

Six MAX values from experiment 3 and eleven MAX values from the short term part of experiment 4 were used. By this method MAX -5 was calculated to be 104 dB SPL and MAX -30 was 79 dB SPL at 6 kHZ.

RESULTS

In this series of experiments changes in the a. c. cochlear potential and the evoked potential of the cochlear nerve (N_1) were studied. The background set of conditions, against which these changes were measured, are shown in Figures 10 through 14. These data are a summary of the control values obtained in this study.

Figures 10, 11 and 12 are frequency histograms of the number of guinea pigs which produced 1 μV of cochlear potential at various sound intensities. A histogram is shown for each frequency routinely measured. These Figures include data from all animals which were used in experiments 1,3 and 4 collected prior to any treatment.

Figures 13 and 14 are frequency histograms of the number of guinea pigs which produced 50 μV of N_1 at various sound intensities. These data were also collected in experiments 1, 3 and 4 before any treatment. They are thought to represent the initial values one would expect to obtain from healthy guinea pigs randomly selected from this inbred strain. Table I is a summary of the median and mean values of the data shown in Figures 10 through 14.

Experiment 1

The effect of sodium salicylate on the cochlea's ability to generate one microvolt of a.c. cochlear potential at various frequencies is shown in Figure 15. These data, which are called the 1 μV frequency function, were obtained six hours after a single subcutaneous dose of sodium salicylate. It can be easily seen that there is no marked change in this frequency function. In fact, if there is any

Figure 10. Frequency histograms of the number of normal guinea pigs producing 1μV of a.c. cochlear potential at various sound intensities. A conversion from dB re: 1 dyne/cm^2 to dB SPL (= re: .0002 dyne/cm^2) can be made by simply adding 74 dB to the dB re: 1 dyne/cm^2.

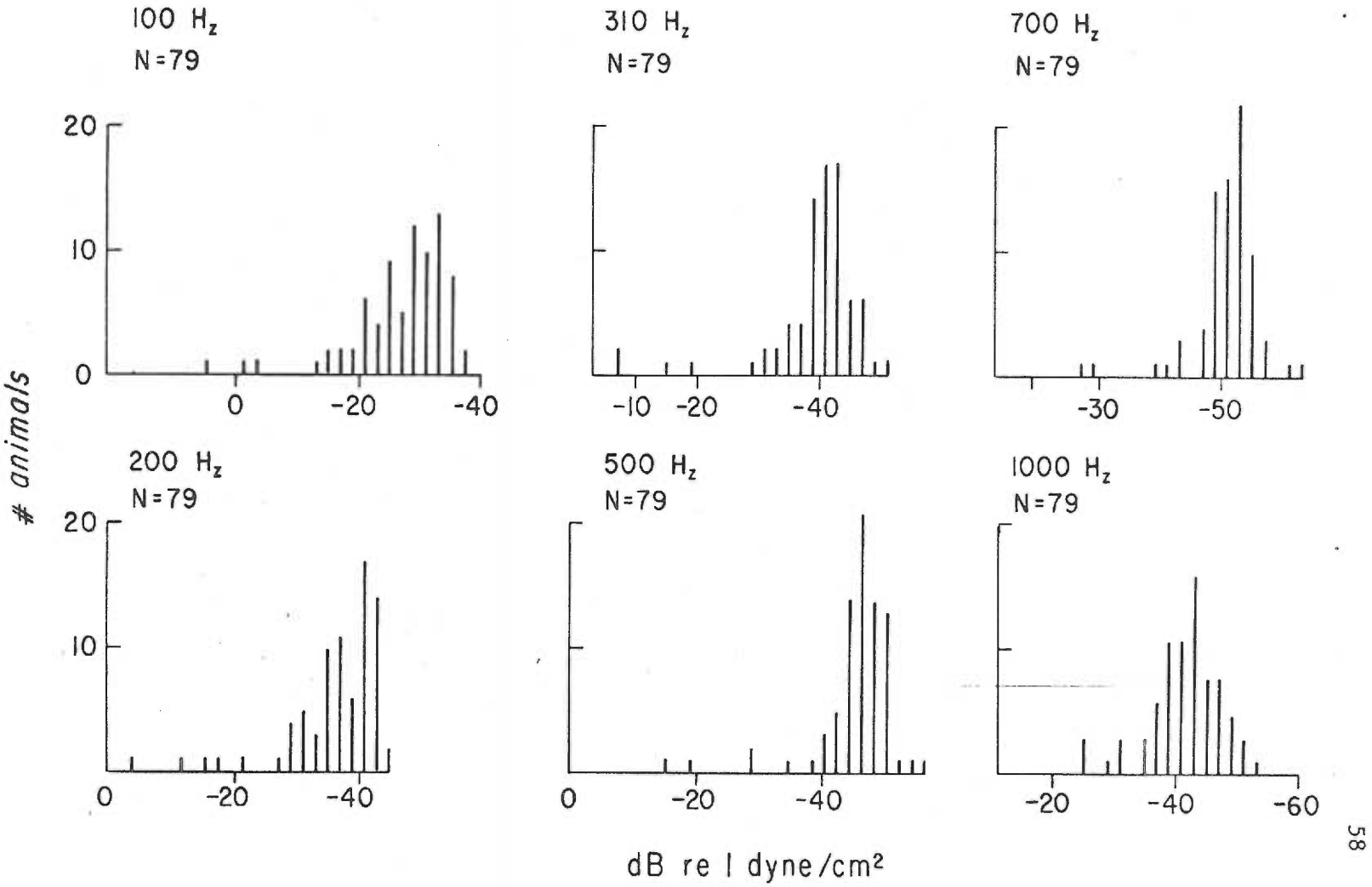

1 μV C.P. Frequency Histograms
100 Hz
N=79
310 Hz
N=79
700 Hz
N=79
200 Hz
N=79
500 Hz
N=79
1000 Hz
N=79
# animals
20
10
0
0
-20
-40
-10
-20
-40
-30
-50
20
10
0
-20
-40
0
-20
-40
-20
-40
-60
dB re 1 dyne/cm²

Figure 11. Frequency histograms of the number of normal guinea pigs producing 1μV of a.c. cochlear potential at various sound intensities. A conversion from dB re: 1 dyne/cm^2 to dB SPL (= re: .0002 dyne/cm^2) can be made by simply adding 74 dB to the dB re: 1 dyne/cm^2.

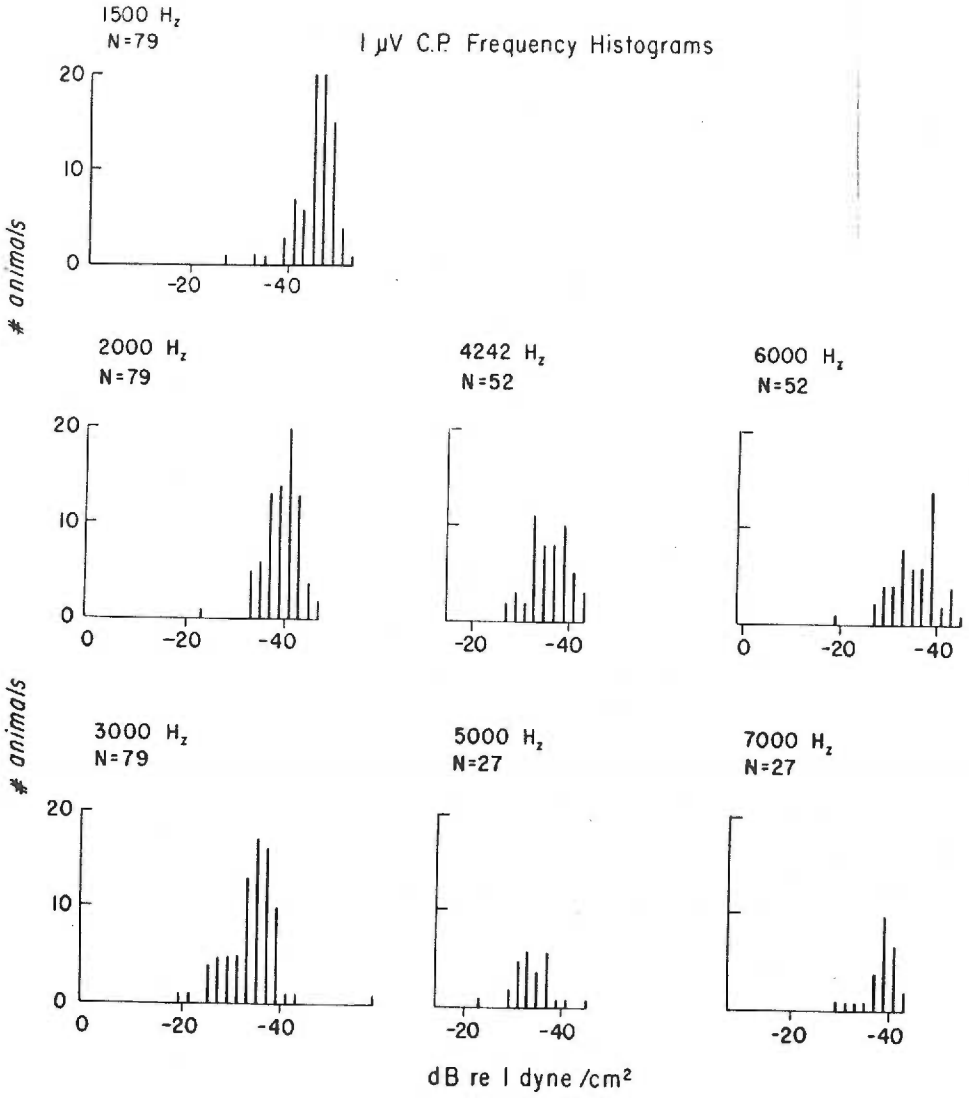

1 μV C.P. Frequency Histograms
1500 Hz
N=79
2000 Hz
N=79
4242 Hz
N=52
6000 Hz
N=52
3000 Hz
N=79
5000 Hz
N=27
7000 Hz
N=27
# animals
# animals
20
10
0
-20
-40
dB re 1 dyne /cm²

Figure 12. Frequency histograms of the number of normal guinea pigs producing 1μV of a.c. cochlear potential at various sound intensities. A conversion from dB re: 1 dyne/cm^2 to dB SPL (= re: .0002 dyne/cm^2) can be made by simply adding 74 dB to the dB re: 1 dyne/cm^2.

1 μV C.P. Frequency Histograms

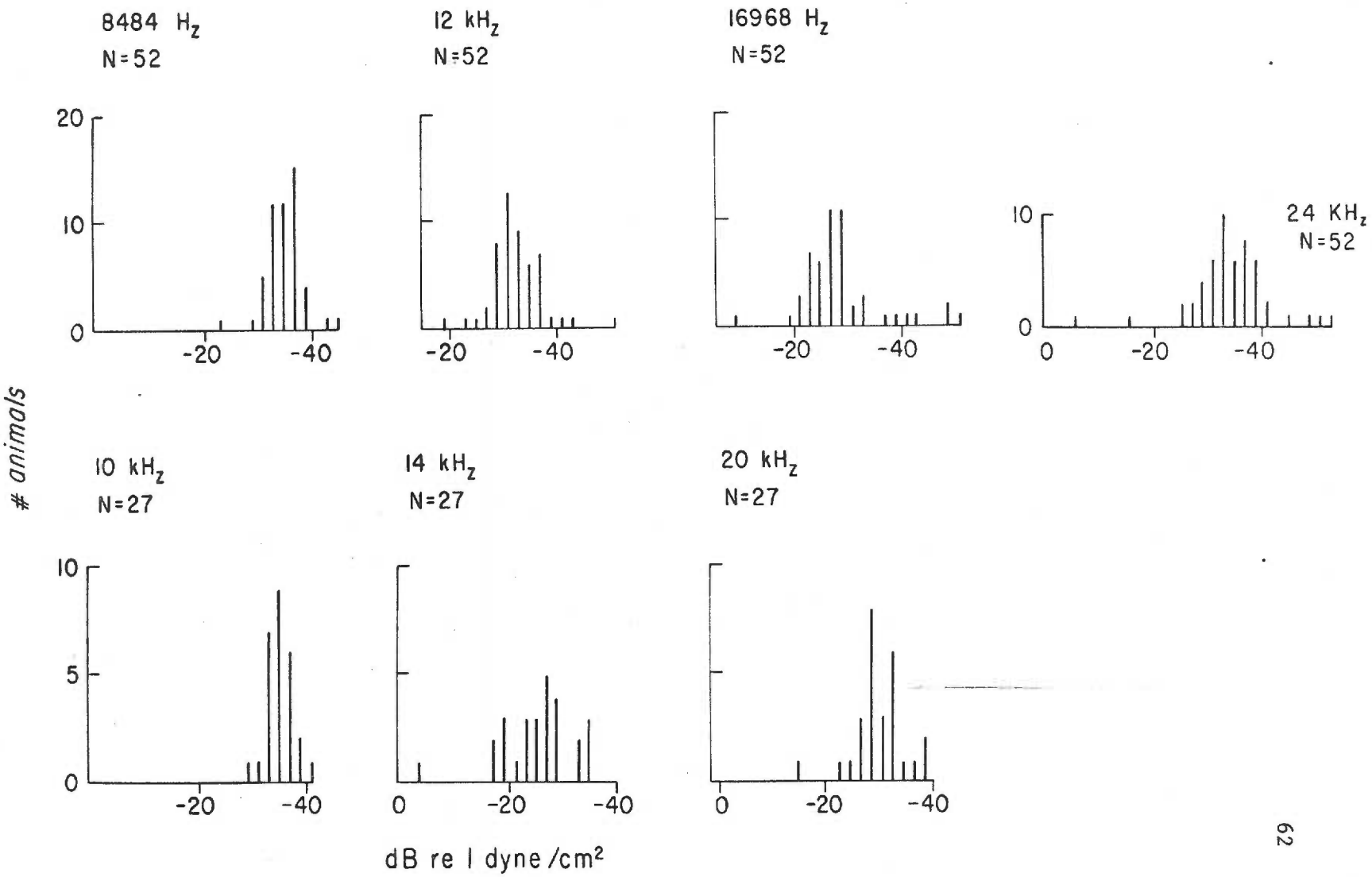


Figure 13. Frequency histograms of the number of normal guinea pigs producing 50μV of N_1 at various sound intensities. A conversion from dB re: 1 dyne/cm^2 to dB SPL (=re: .0002 dyne/cm^2) can be made by simply adding 74 dB to the dB re: 1 dyne/cm^2.

N_1 Frequency Histograms

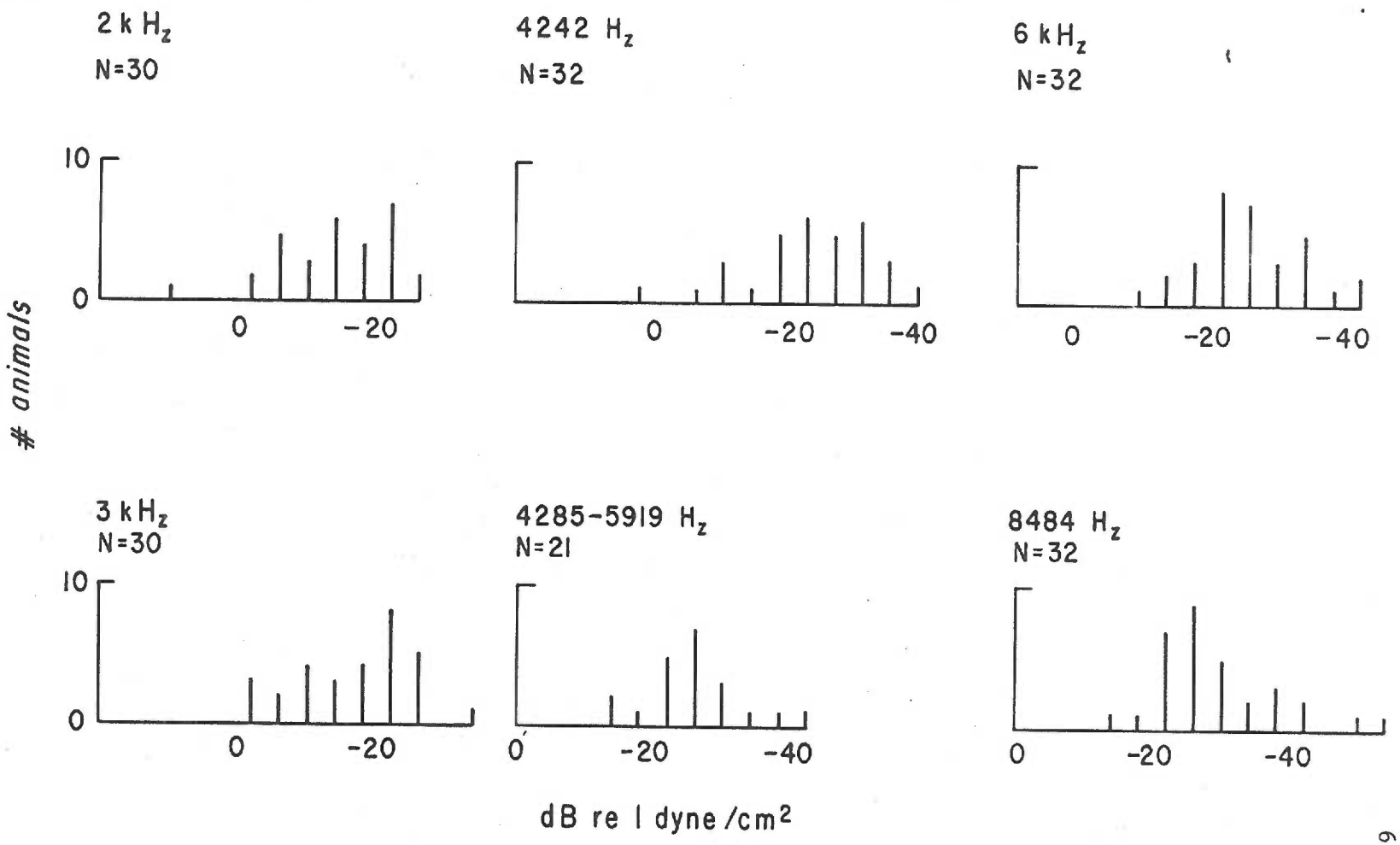


Figure 14. Frequency histograms of the number of normal guinea pigs producing 50 μV of N_1 at various sound intensities. A conversion from dB re: 1 dyne/cm^2 to dB SPL (= re: .0002 dyne/cm^2) can be made by simply adding 74 dB to the dB re: 1 dyne/cm^2.

N_1 Frequency Histograms

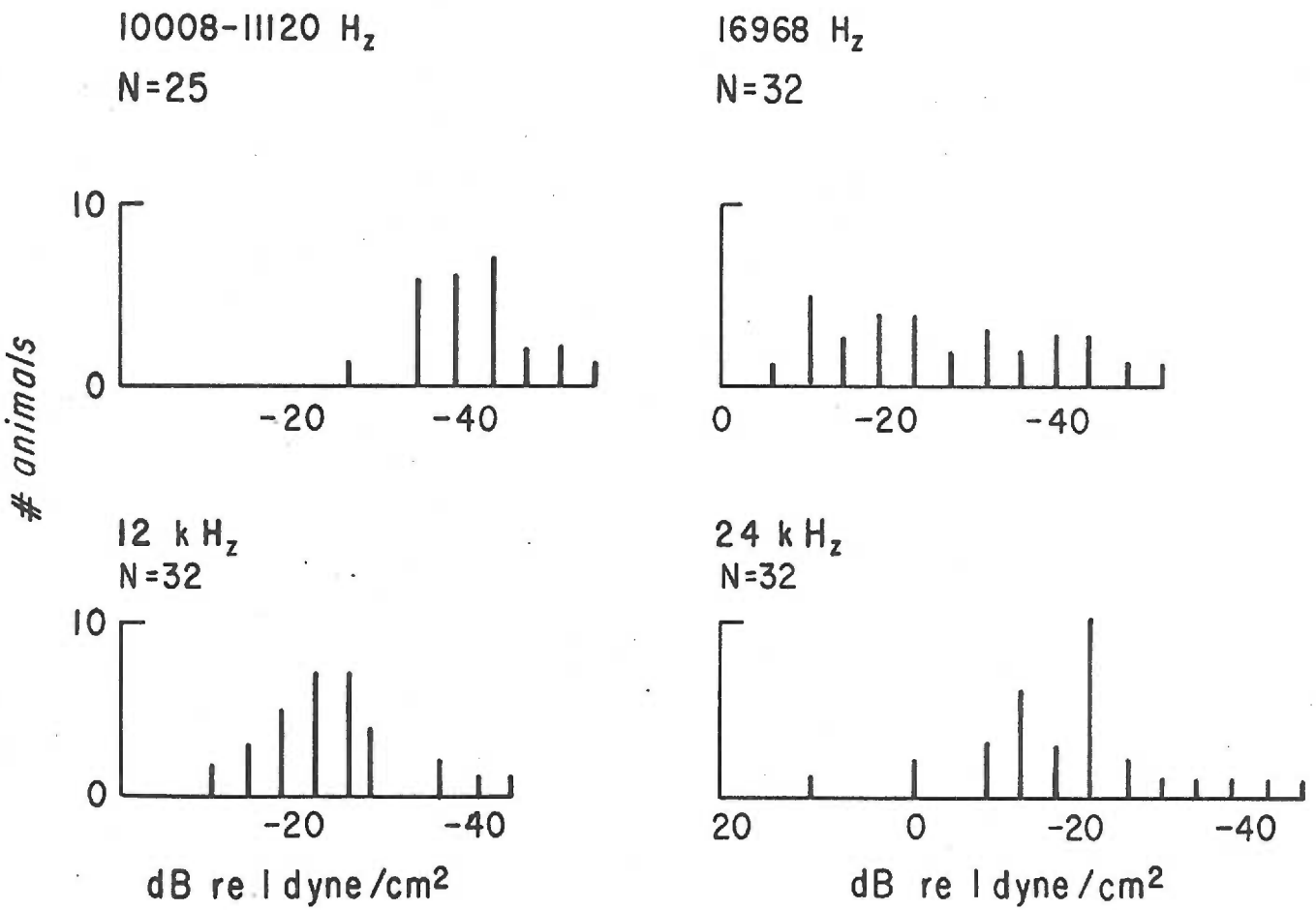
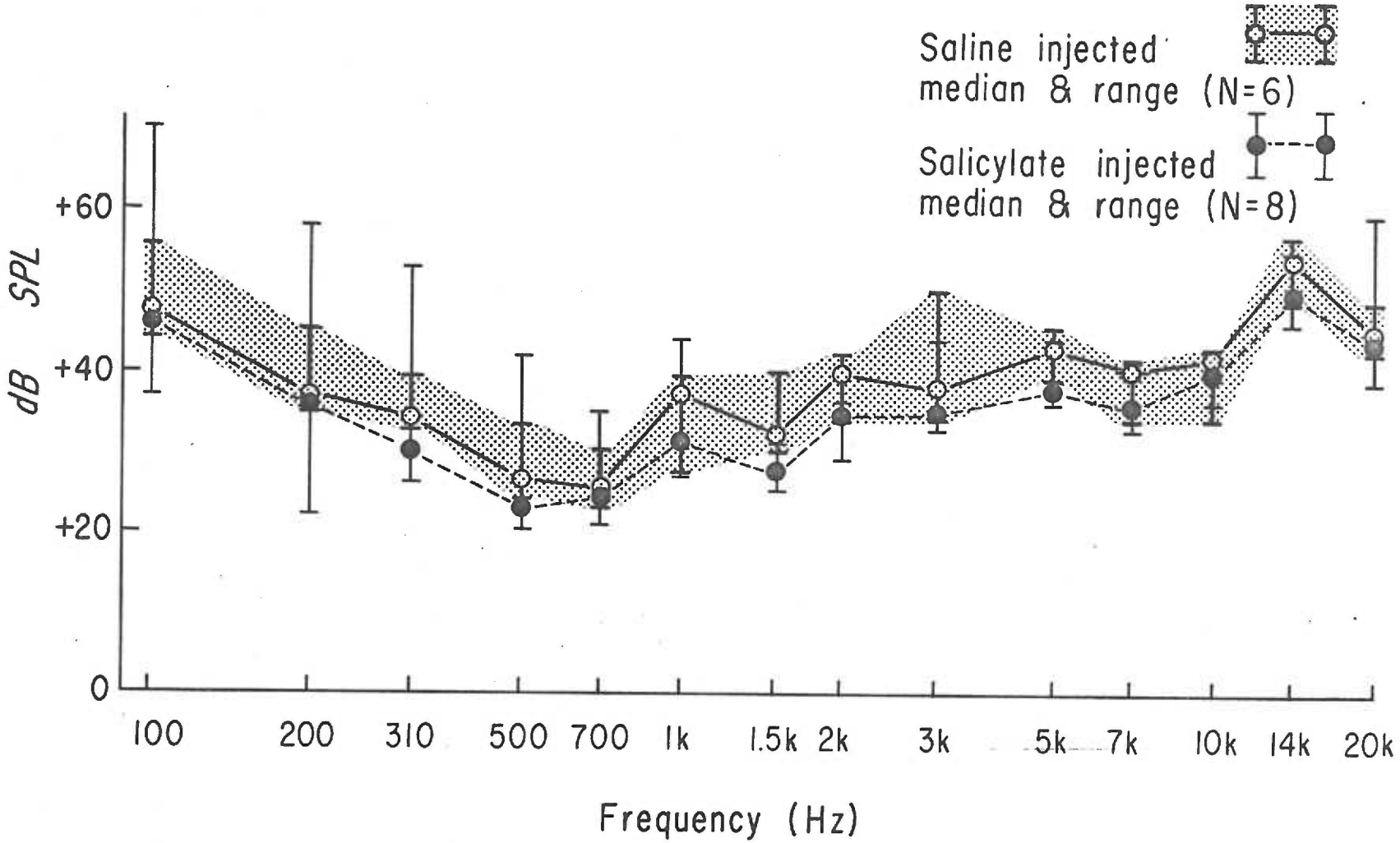


Table I. A summary of the data contained in the histograms in Figures 10 through 14. The median, mean and standard deviation of the histograms at each frequency are shown. A conversion from dB re: 1 dyne/cm^2 to dB SPL (= re: .0002 dynes/cm^2) can be made by adding 74 dB to the dB re: 1 dyne/cm^2.

Frequency (Hz)	dB re: 1 dyne/cm^2 to produce 1 μV cochlear potential			dB re: 1 dyne/cm^2 to evoke 50 μV of N_1		
	Median	Mean	Standard Deviation	Median	Mean	Standard Deviation
100	-29.0	-27.0	8.98			
200	-38.0	-36.6	8.53			
310	-42.0	-40.1	7.56			
500	-48.0	-46.6	6.47			
700	-52.0	-51.3	5.30			
1000	-43.0	-42.0	5.91			
1500	-47.0	-45.9	4.17			
2000	-40.0	-37.9	3.93	-14.0	-14.1	8.21
3000	-35.0	-34.7	5.42	-19.0	-17.6	8.26
4242	-36.5	-36.3	3.98	-24.0	-22.9	8.57
4285-5919				-27.0	-26.4	6.53
5000	-34.0	-34.5	4.32			
6000	-37.5	-36.4	4.86	-25.5	-26.5	7.33
7000	-40.0	-39.0	3.13			
8484	-35.0	-35.6	3.42	-28.0	-30.0	8.87
10k	-36.0	-35.6	2.45			
10008-11120				-39.0	-40.0	6.32
12k	-32.5	-33.2	4.94	-23.0	-24.7	8.13
14k	-27.0	-25.8	6.84			
16968	-28.0	-29.3	7.52	-23.5	-25.3	12.30
20k	-30.0	-30.7	4.82			
24k	-34.5	-34.6	7.53	-21.0	-19.8	11.30

Figure 15. The 1 μV cochlear potential frequency function for salicylate (both 286 and 545 mg/kg) and saline injected guinea pigs. These values were obtained 6 hours after injection in both groups when the N_1 depression was maximal. The slight increase in the cochlear potential is not thought to be biologically significant. See Figure 26 for more data relevant to this point. These animals are also shown in Table II.


Saline injected
median & range (N=6)
Salicylate injected
median & range (N=8)
dB SPL
+60
+40
+20
0
100
200
310
500
700
1k
1.5k
2k
3k
5k
7k
10k
14k
20k
Frequency (Hz)

change, it is to enhance the 1 μV a. c. cochlear potential. The enhancement here is not thought to be a significant one and may have been due to a slightly higher body temperature in the salicylate injected group due to the standardized heating procedures.

Changes occurring in the a. c. cochlear potential and N_1 during a six hour period after a single subcutaneous dose of sodium salicylate are shown in Figures 16 and 17. In addition, the blood concentration of salicylate is shown over the same time course. Only mean data for all three of these parameters are shown. The plot of the a. c. cochlear potential shows increases or decreases in the amount of sound that was required to produce one microvolt of a. c. cochlear potential. These data were obtained at the same frequency as that of the tone pulses that were used to generate N_1. Figure 16 shows the data obtained at frequencies between 4850 Hz and 4900 Hz and is called the middle frequency plot. Figure 17 shows the results for the high frequencies. Changes from initial control values in the amount of sound required to produce 50 microvolts of peak-to-peak amplitude in N_1 are shown for the same time course as is the a. c. cochlear potential. It was felt that this is justified as representative of the change in N_1, because the change in the intensity function of N_1 was approximately the same at 25 μV, 50 μV, 100 μV and 150 μV (See Figure 7).

There are some slight changes in the amount of sound required to produce N_1 and the cochlear potential in the saline group. However, in

Figure 16. The results from the short term part of Experiment 1. Mean changes in the sound required to produce 1 microvolt of a. c. cochlear potential and 50 microvolts of N_1 during the six hour period after sodium salicylate (545 mg/kg) or isotonic saline (5 ml/kg) are shown. The most noticable effect is that during the six hour time period the animals which received sodium salicylate required an ever increasing amount of sound to produce a 50 μV N_1 response. The variability of these measures can be estimated from the individual data shown in Table II. The cochlear potential values at the 2, 4 and 5 hour time periods are based on interpolated values from recordings made at 1, 3 and 6 hours after injection.

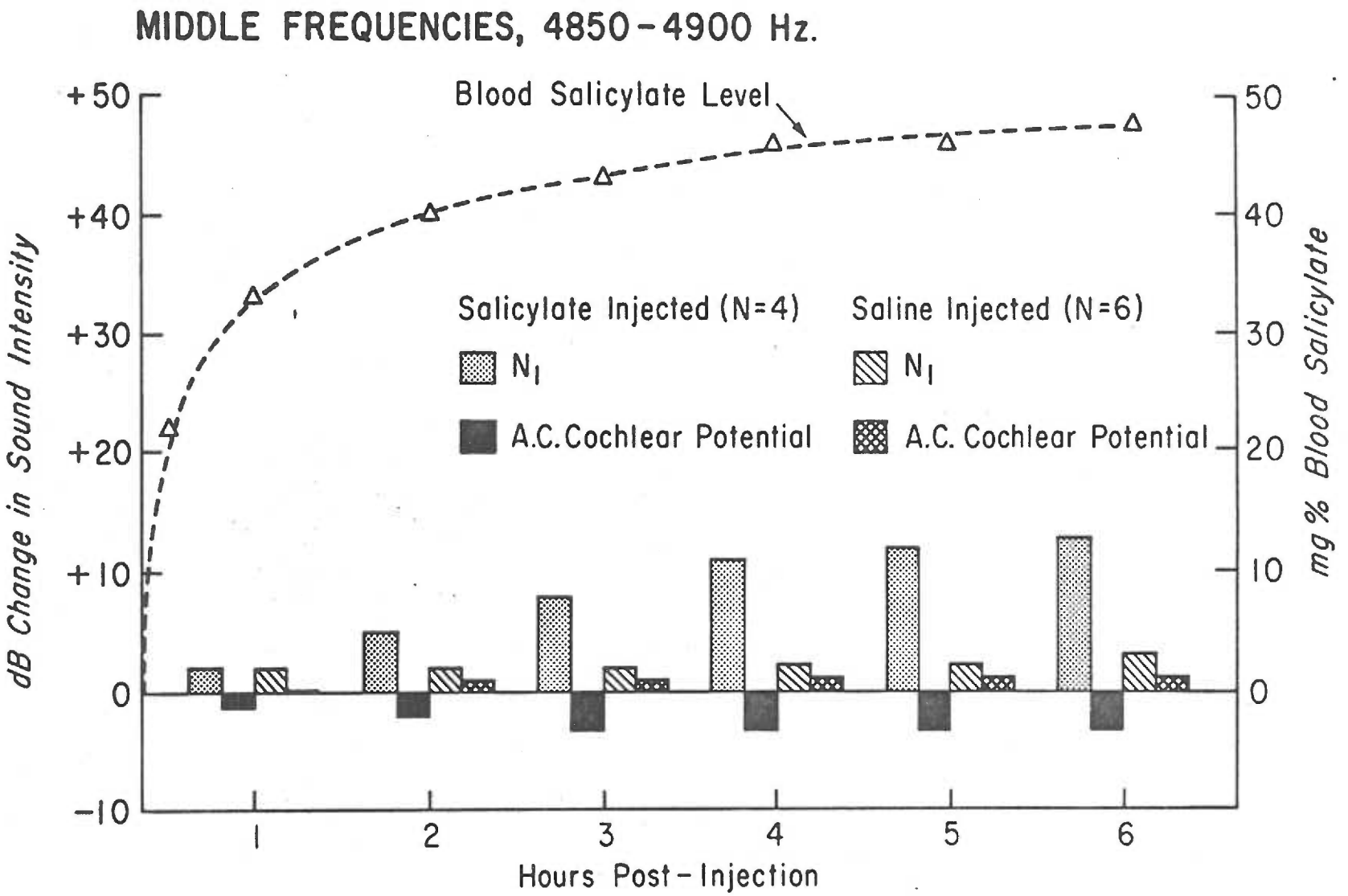
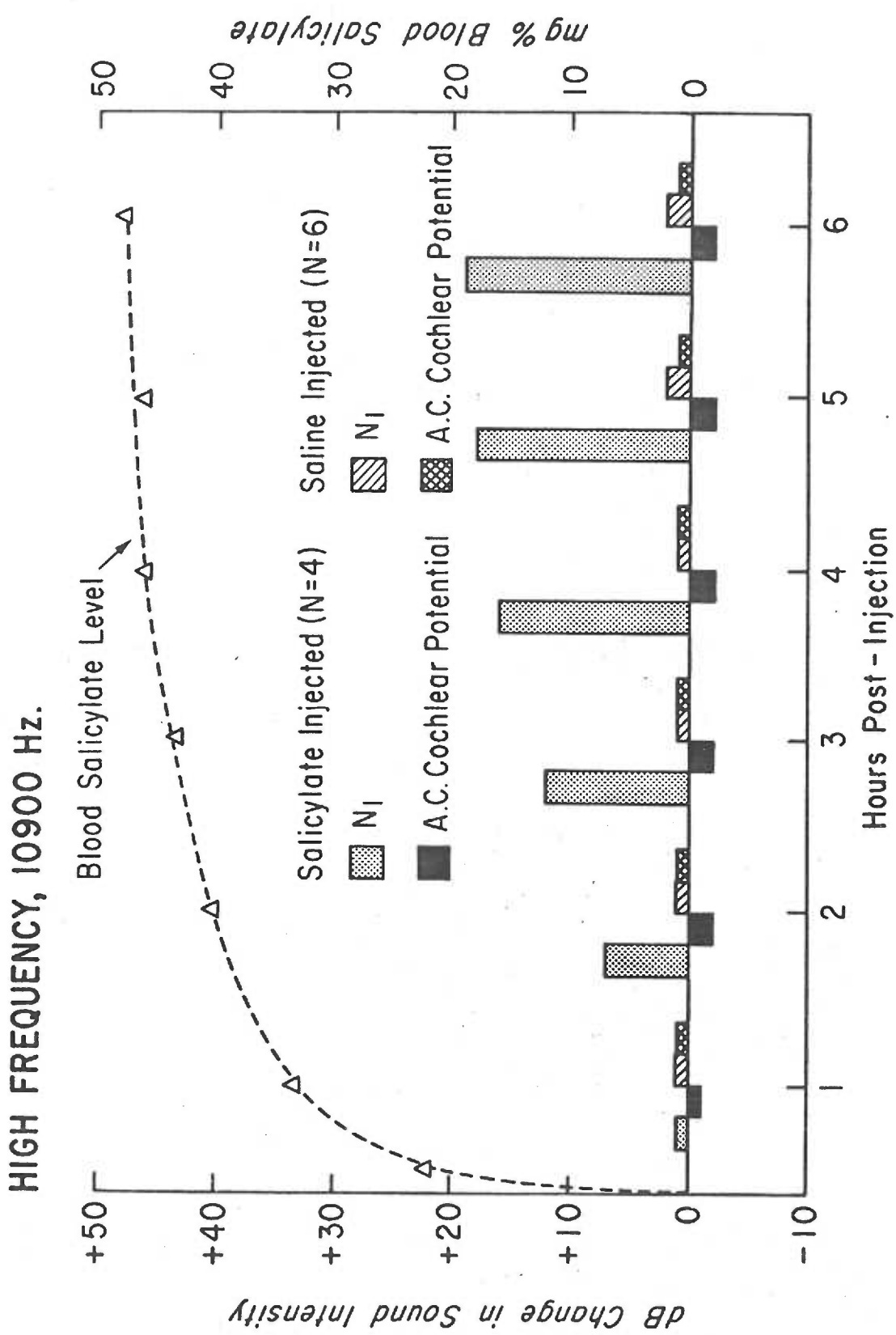

MIDDLE FREQUENCIES, 4850-4900 Hz.
Blood Salicylate Level
Salicylate Injected (N=4)
Saline Injected (N=6)
N1
A.C. Cochlear Potential
N1
A.C. Cochlear Potential
+50
+40
+30
+20
+10
0
-10
50
40
30
20
10
0
1
2
3
4
5
6
Hours Post-Injection
dB Change in Sound Intensity
mg % Blood Salicylate

Figure 17. The results from the short term part of Experiment 1. Mean changes in the sound required to produce 1 microvolt of a. c. cochlear potential and 50 microvolts of N_1 during the six hour period after sodium salicylate (545 mg/kg) or isotonic saline (5 ml/kg) are shown. The most noticable effect is that during the six hour time period the animals that received sodium salicylate required an ever increasing amount of sound to produce a 50 μV N_1 response. The variability of these measures can be estimated from the individual data shown in Table II.

The cochlear potential values at the 2, 4 and 5 hour time periods are based on interpolated values from recordings made at 1, 3 and 6 hours after injection.


HIGH FREQUENCY, 10900 Hz.
Blood Salicylate Level
Solicylate Injected (N=4)
N1
A.C. Cochlear Potential
Saline Injected (N=6)
N1
A.C. Cochlear Potential
+50
+40
+30
+20
+10
0
-10
dB Change in Sound Intensity
50
40
30
20
10
0
mg % Blood Solicylate
1
2
3
4
5
6
Hours Post-Injection

no case did the change exceed 9 dB. A slight decrease in the amount of sound required to produce one microvolt of a.c. cochlear potential occurred in the salicylate treated animals. The most noticeable effect is that during the six hour time period the animals which received the sodium salicylate required an ever increasing amount of sound to produce a 50 μV N_1 response. This effect was noticeable at the middle frequencies as well as the high frequencies. It can be noted that a one or two hour time lag exists between the accumulation of salicylate in the blood and the observed decrement of N_1. In order to show the variability of these observations, data from individual animals are shown in Table II. The effects of the low dose (286 mg/kg) at six hours after injection is significant beyond the .05 level (Kolmogorov-Smirnov test, 8, 62, 82) and the high dose changes in N_1 are significant beyond the .005 level (Kolmogorov-Smirnov test).

Data from the long term part of this experiment in which the sodium salicylate or saline was injected subcutaneously and then the animals were maintained five, eight, twenty four or 168 hours before being evaluated are shown in Figure 18. The mean data for the saline injected animals at each time period are represented as the zero line. Increases in the amount of sound required to produce 50 μV of N_1 are shown relative to the zero line. Blood concentrations of salicylate observed at these time intervals are also shown. It can be seen that the maximum effect on N_1 occurs between five and eight hours, that it has nearly recovered in 24 hours and is within normal

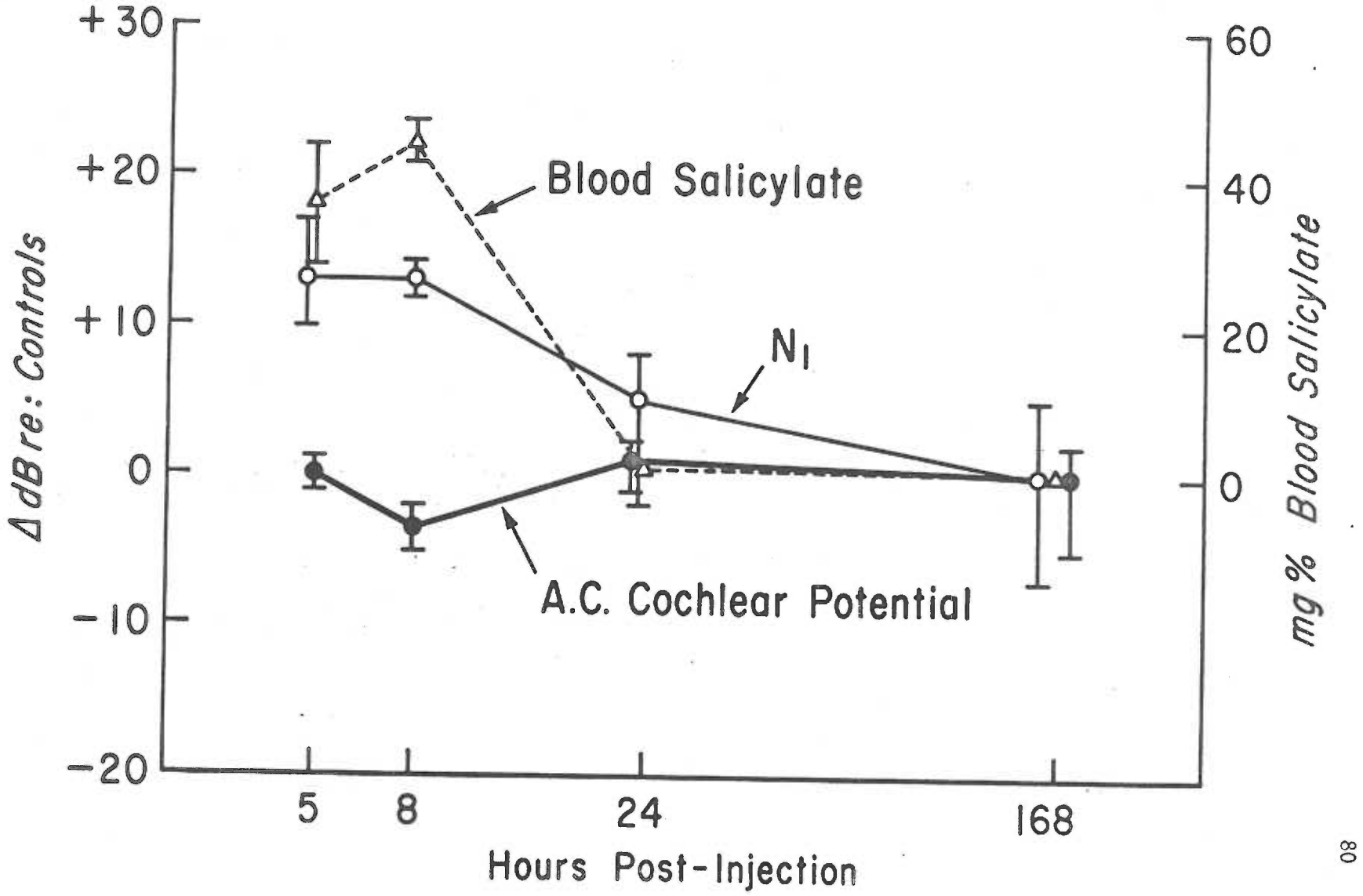
Table II. The data from the short term part of Experiment 1 demonstrating the decrement in N_1 and no appreciable change in the a. c. cochlear potential from two different dosages of sodium salicylate. The electrophysiological data represent changes in the amount of sound (dB) from initial values which were necessary to obtain the specified amount of the a. c. cochlear potential* of N_1**.

For example, consider the data for animal 323-A, which at 4285 Hz showed a slight improvement (0, -1, -2 dB) in the cochlear potential. On the other hand when the N_1 measures before injection were compared with those after injection there was a loss (-1, +3, +11).

Animal No.	Salicylate Dosage (mg/kg)	Stimulus Frequency (Hz)	CP* Hours post-injection 1 3 6 (dB change)	N_1** Hours post-injection 1 3 6 (dB change)	Stimulus Frequency (Hz)	CP* Hours post-injection 1 3 6 (dB change)	N_1** Hours post-injection 1 3 6 (dB change)	Blood salicylate. Hours post-injection 1 3 6 (mg %)
319-A	0	5919	0 0 +2	0 +4 +4	10008	-2 -2 -1	-1 0 +2	- - -
324-A	0	5000	0 0 +2	+5 +2 +5	10900	+1 +2 +3	0 -6 -5	- - -
327-A	0	4850	-1 -2 -3	+2 0 +2	10965	0 -1 -2	+1 -1 +1	- - -
331-A	0	4850	+1 +3 0	+2 +4 +4	10900	+4 +7 +5	+3 +7 +9	- - -
335-A	0	4900	+1 +3 +3	0 +1 0	10900	+1 +2 +1	0 -1 +2	- - -
338-A	0	4800	0 +1 0	+3 +4 +5	10800	0 -1 -1	+1 +2 +3	- - -
312-A	286	5919	-1 0 -1	0 +4 +16	10008	+3 +6 +8	+2 +7 +23	@ @ 33
313-A	286	5919	-1 -2 -5	-10 -8 -8	10008	+3 +5 +5	-6 +1 0	26 26 25
323-A	286	4285	0 -1 -2	-1 +3 +11	11120	0 0 -2	+1 +7 +14	20 28 28
326-A	286	4850	-1 -2 -4	-2 +2 +4	10950	0 0 -2	-2 +2 +7	5 10 13
332-A	286	4850	-1 -2 -3	0 +7 +12	10900	0 +1 +1	+2 +11 +17	20 29 31
334-A	545	4850	-1 -2 -3	+2 +4 +8	10900	0 0 -1	+1 +6 +13	29 44 48
336-A	545	4900	-1 -2 -3	+3 +11 +15	10900	-1 -2 -2	+1 +15 +21	34 40 44
339-A	545	4800	-1 -4 -4	+2 +8 +14	10900	-1 -4 -4	+2 +13 +22	30 43 49
340-A	545	4850	-2 -4 0#	+3 +11 +18	10900	-1 -2 0#	0 +15 +20#	40 44 50

* 1 μV a. c. cochlear potential. ** 50 μV N_1. @ Data lost. # from 5 1/2 hour time

Figure 18. Results of the long term part of Experiment 1 demonstrating recovery from the salicylate effect. The stimulating frequency was 10 kHz for both the cochlear potential and N_1. At each of the specified times the mean and range of 3 or 4 salicylate injected animals are compared with the mean of two control animals. Increases in the amount of sound required to produce 50 μV of N_1 are shown relative to the zero line. A total of 22 animals were used in this part of Experiment 1. One experimental animal is not included in these data because, no N_1 responses could be elicited even though the cochlear potential was normal. If this animal had been included, it would have increased the difference between the control and experimental groups.


+30
+20
+10
0
−10
−20
60
40
20
0
Δ dB re: Controls
mg % Blood Salicylate
Blood Salicylate
N1
A.C. Cochlear Potential
5
8
24
168
Hours Post-Injection

limits by 168 hours after injection. Only a small change in the cochlear potential is seen at any time and again this is in the direction of an augmentation. It can easily be seen that the effect of a single subcutaneous injection of sodium salicylate on N_1 is reversible.

Experiment 2: Cochlear Perfusion

Initial development of the cochlear perfusion technique used in this experiment was accomplished by Dr. Robert Brummett (9). In utilizing this technique the initial goal was to place two pipets, as shown in Figure 9, and to perfuse the cochlea without affecting the cochlear potential. Thirteen guinea pigs were used in these initial attempts. The process of drilling two small holes (0.012 inch in diameter) in the cochlea, placing the pipets, and perfusing the cochlea with synthetic perilymph without changing the a. c. cochlear potential more than 6 dB was successful in five of the thirteen attempts.

In the initial attempts N_1 was not systematically measured but it was observed to be very easily damaged, and if the a. c. cochlear potential was reduced equivalent to 10 dB the N_1 decrements were greater than 30 dB. Thus a refinement of the perfusion technique was needed.

A second goal in utilizing this technique was to obtain perfusions while retaining an acceptable N_1 response. Therefore data was only accepted from animals which had less than a 20 dB decrement in N_1 after pipet placement and one perfusion with synthetic perilymph.

Ten additional guinea pigs were used and five of these met this new criteria. These cochleas were then perfused with synthetic perilymph containing sodium salicylate (20 to 40 mg%) and the cochlear potential and N_1 were again recorded. Following these recordings the cochleas were perfused again with synthetic perilymph, rinsed, in order to remove the sodium salicylate. The procedure of making these three perfusions and the appropriate recordings, shown in Figure 19, took two to two and a half hours. The most striking feature is that the second perfusion, containing the salicylate, produced a decrement in N_1 and very little change in the a. c. cochlear potential, and that this effect was reversed by the third perfusion, a rinse with synthetic perilymph not containing sodium salicylate.

These concentrations of 20 to 40 mg% of sodium salicylate can be compared with the perilymph concentrations reported by Silverstein et al. (83). They reported an average level of 25 mg% in the perilymph when the blood levels were reported to be 69 mg%.

Experiment 3: The Effects of Intense Sound

The effects of intense pure tone stimulation upon N_1 was evident only at some frequencies. The depression of N_1, at a given frequency, was uniform up to about 100 μV. Therefore, the data shown in Figures 20 through 23 are plots of the change in the sound intensity necessary to produce 50 μV of N_1. In addition the 1 μV a. c. cochlear potential is also presented. An increase in the amount of sound necessary to produce 50 μV of N_1 is interpreted as a loss or decrement in N_1.

The data in Figure 20 are for individual animals exposed to

Figure 19. The results of Experiment 2 demonstrate a decrease and then a recovery of N_1 from sodium salicylate. The changes in the 50 μV level of N_1 and the 1 μV a. c. cochlear potential, from initial values, are shown for four individual animals. Recordings were made after the following procedures: 1. After drilling two holes in the cochlea, placing two pipets and perfusing once with synthetic perilymph. 2. After the second perfusion, with sodium salicylate added to the perilymph. 3. After the third perfusion with synthetic perilymph alone. The animals were perfused as follows: # 211-A was perfused with 20 mg%. # 212-A was perfused with 30 mg%. Animals # 214-A, 467-A and 482-A were perfused with 40 mg%.

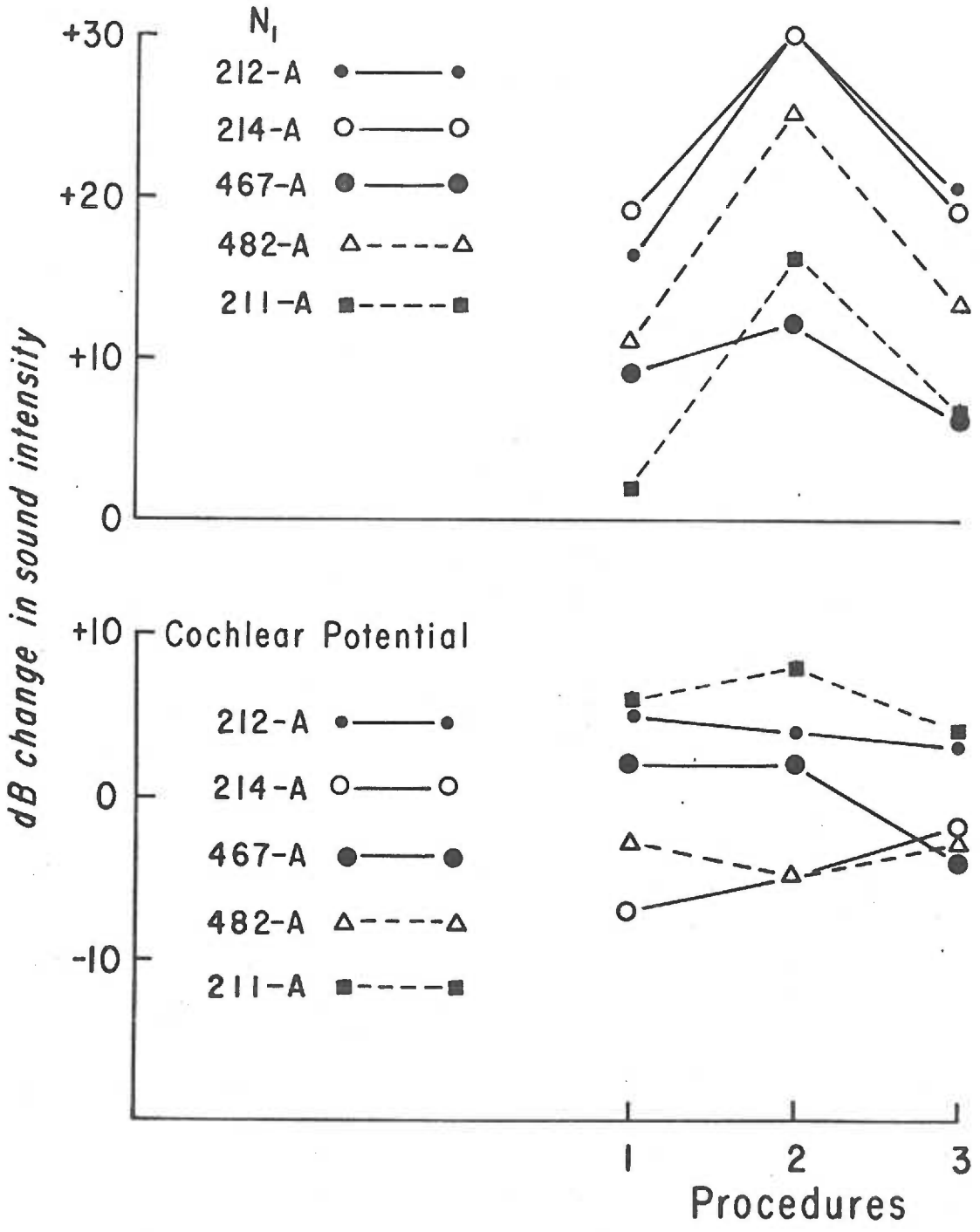

N1
212-A
214-A
467-A
482-A
211-A
+30
+20
+10
0
dB change in sound intensity
Cochlear Potential
+10
0
-10
1
2
3
Procedures

Figure 20. The results of stimulation with a 3 kHz tone for 15 minutes (indicated by the arrow). The changes, from preexposure, in the sound required to produce 1 microvolt of the a. c. cochlear potential or 50 microvolts of N_1 are plotted. These data are for individual animals, four experimental (MAX -5) and two control (MAX -30) animals are shown. The experimental animals show a maximum shift in N_1 at half an octave above the exposure frequency.

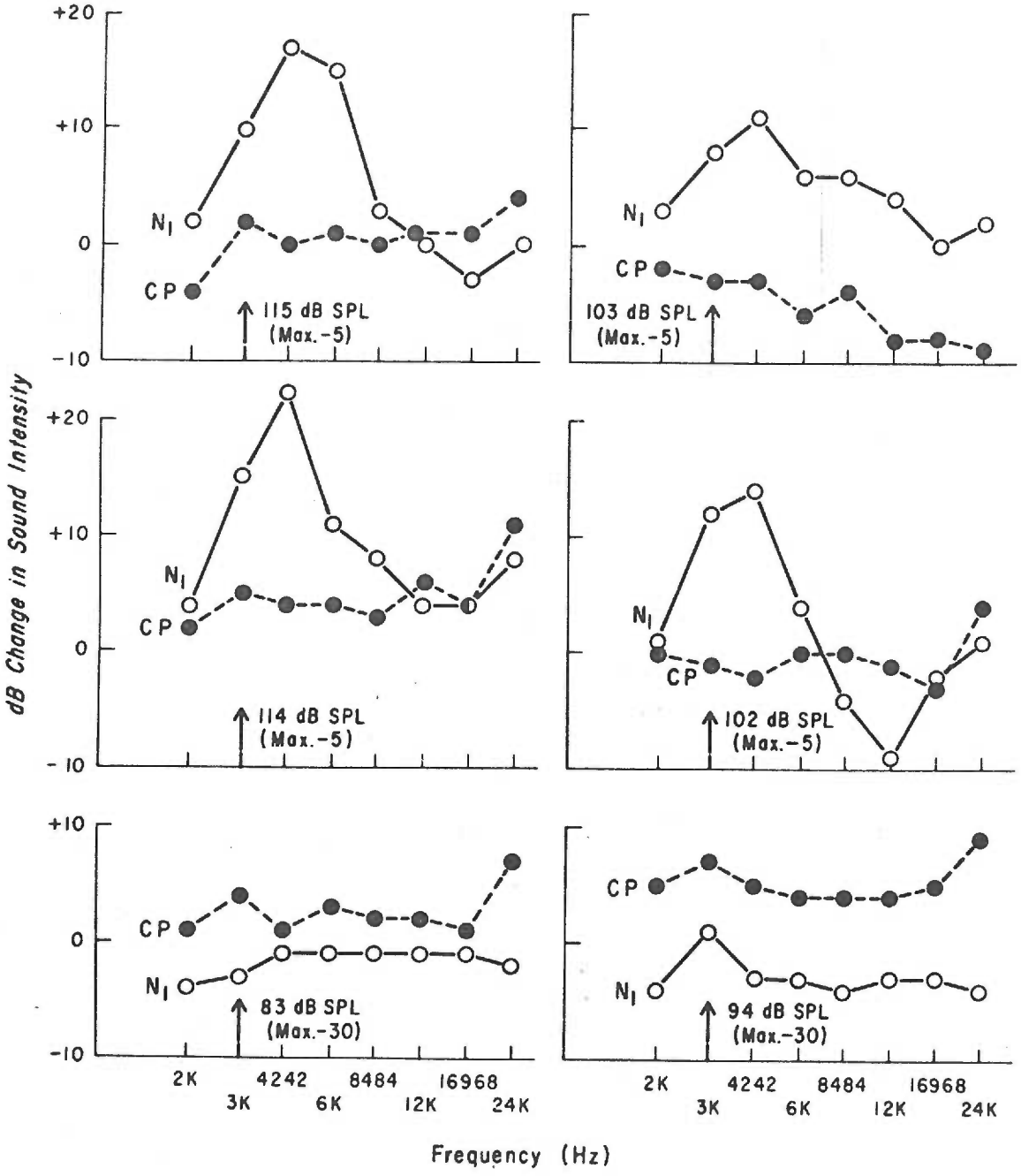

+20
+10
0
-10
N1
CP
115 dB SPL
(Max.-5)
103 dB SPL
(Max.-5)
114 dB SPL
(Max.-5)
102 dB SPL
(Max.-5)
83 dB SPL
(Max.-30)
94 dB SPL
(Max.-30)
dB Change in Sound Intensity
2K
3K
4242
6K
8484
12K
16968
24K
Frequency (Hz)

Figure 21. The results of stimulation with a 6 kHz tone for 15 minutes (indicated by the vertical arrow). The changes, from pre-exposure, in the sound required to produce 1 microvolt of the a. c. cochlear potential or 50 microvolts of N_1 are plotted. These data are for individual animals, four experimental (MAX -5) and two control (MAX -30) animals are shown.

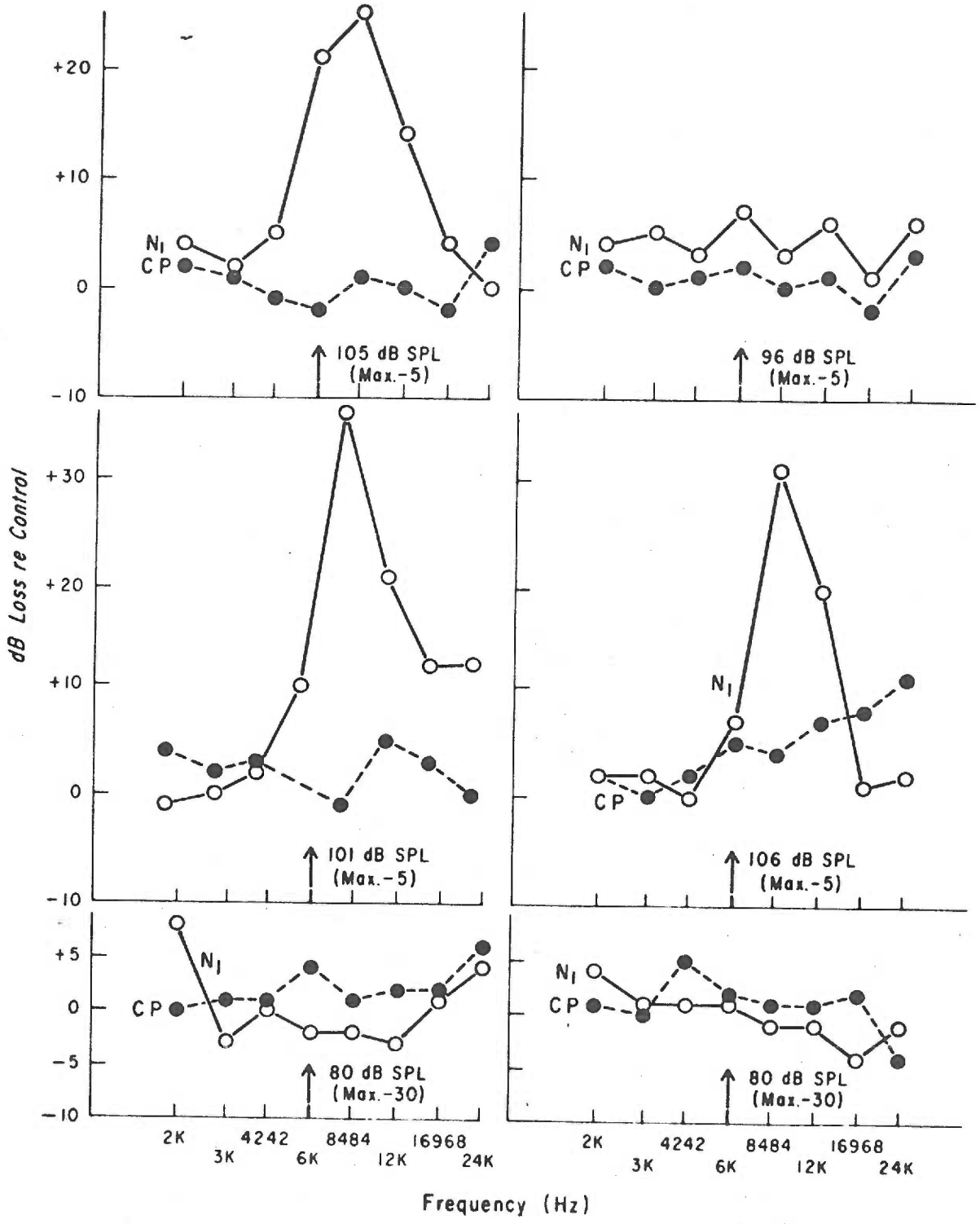
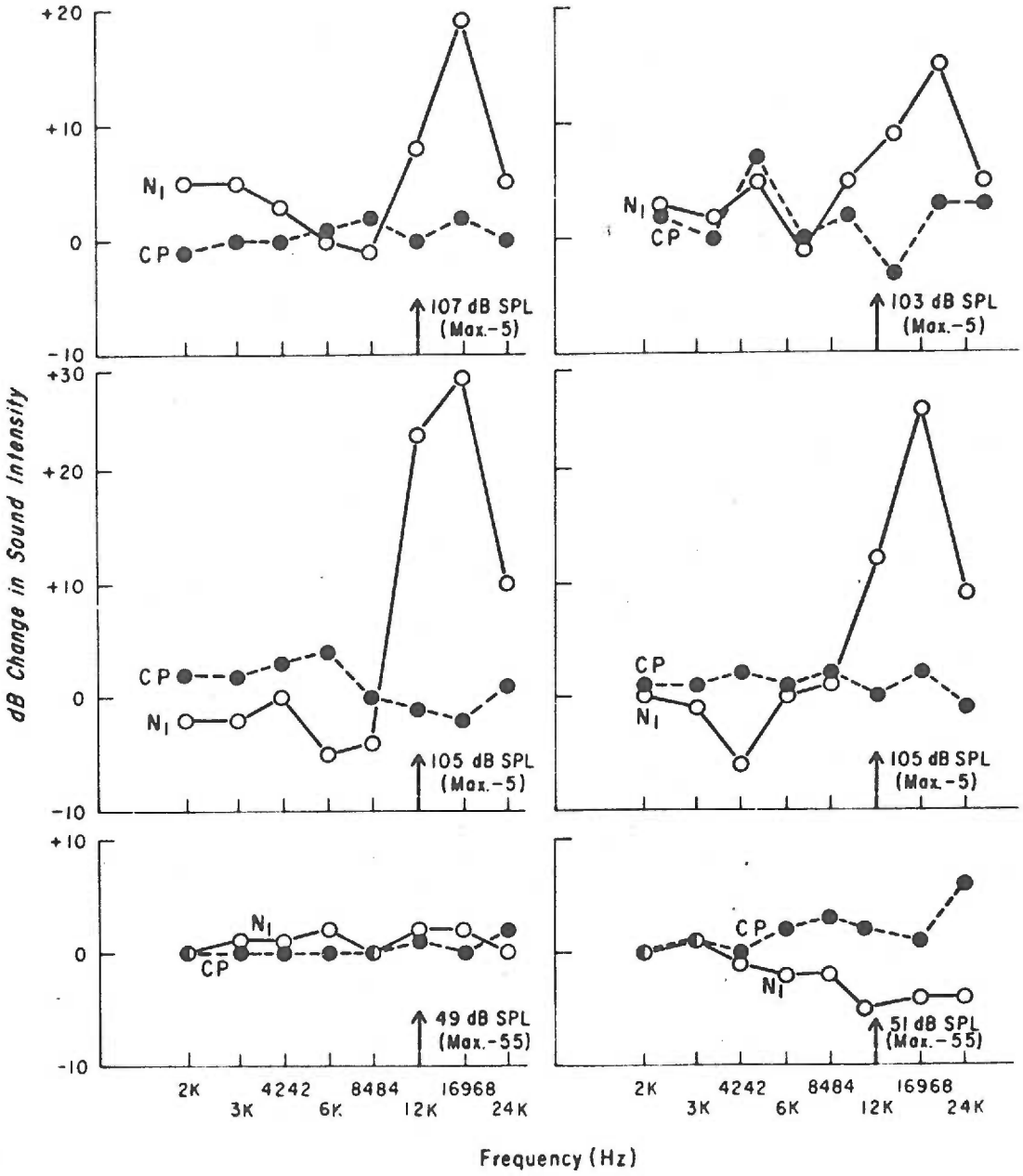

+20
+10
0
-10
N1
CP
105 dB SPL
(Max.-5)
96 dB SPL
(Max.-5)
dB Loss re Control
+30
+20
+10
0
-10
101 dB SPL
(Max.-5)
106 dB SPL
(Max.-5)
+5
0
-5
-10
80 dB SPL
(Max.-30)
2K
3K
4242
6K
8484
12K
16968
24K
Frequency (Hz)

Figure 22. The results of stimulation with a 12 kHz tone for 30 minutes (indicated by the vertical arrow). The changes, from pre-exposure, in the sound required to produce 1 microvolt of a. c. cochlear potential or 50 microvolts of N_1 are plotted. These data are for individual animals, four experimental (MAX -5) and two control (MAX -55) animals are shown.


+20
+10
0
-10
+30
+20
+10
0
-10
+10
0
-10
dB Change in Sound Intensity
N1
CP
107 dB SPL
(Max.-5)
103 dB SPL
(Max.-5)
105 dB SPL
(Max.-5)
105 dB SPL
(Max.-5)
49 dB SPL
(Max.-55)
51 dB SPL
(Max.-55)
2K
3K
4242
6K
8484
12K
16968
24K
Frequency (Hz)

3 kHz for 15 minutes. Four experimental animals which were stimulated at MAX -5 and two control animals, stimulated at MAX -30, are shown. In each of the animals exposed to MAX -5 the pattern of loss in N_1 was similar to reported hearing losses. That is, the maximum loss in N_1 was half an octave above 3 kHz, at 4242 Hz, and little or no loss was found below 3 kHz, at 2 kHz. At the same time, there was no appreciable change in the amount of sound needed to produce 1 μV of a.c. cochlear potential at any frequency. In addition to the lack of change in the 1 μV level, no change was found in the a.c. cochlear potential intensity functions recorded at the stimulating frequency, 3 kHz, and half an octave above it, at 4242 Hz.

The data for individual animals exposed to 6 kHz for 15 min. are shown in Figure 21. Again, four animals were stimulated at MAX -5 and two were stimulated at MAX -30. The experimental animals, stimulated at MAX -5, show a pattern of N_1 loss similar to reported hearing losses while the cochlear potential again showed no appreciable change. This was also true for cochlear potential intensity functions recorded at 6 kHz and 8484 Hz.

In Figure 21 it can be seen that one experimental animal, exposed at MAX -5, did not show any appreciable loss in N_1. Some variations between individual animals is to be expected and it can be seen that the absolute level of MAX -5, that is 96 dB SPL, was slightly less than the MAX -5 point of the other experimental animals. Thus, this animal in terms of sound pressure level, was stimulated at an intensity between the other three experimental animals

and the control animals. Thus, the lack of an N_1 change is not illogical.

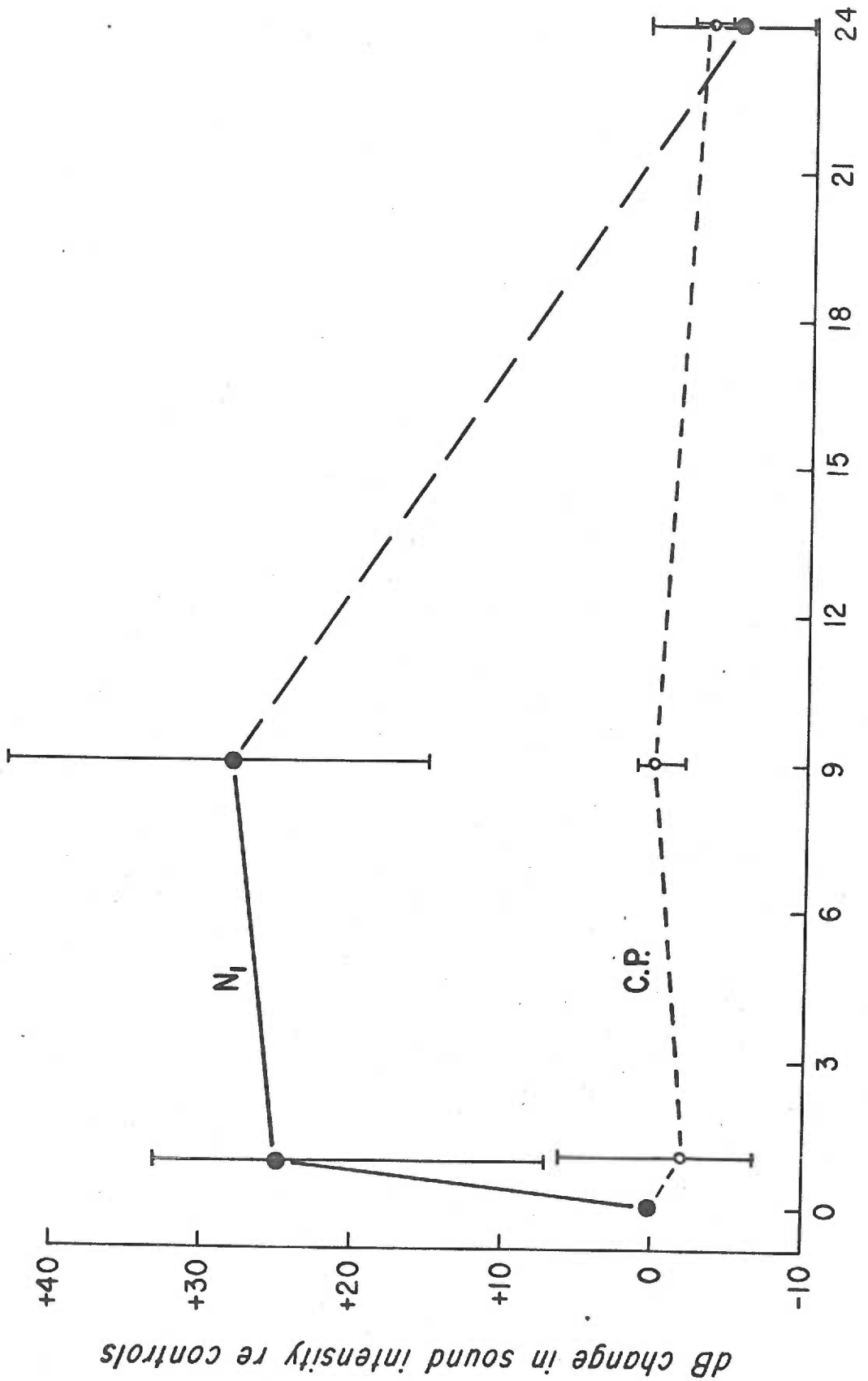
The data for individual animals exposed to 12 kHz for 30 minutes are shown in Figure 22. The four experimental animals were stimulated at MAX -5 and two controls were stimulated at MAX -55. Animals which were stimulated at MAX -30 showed a slight loss in N_1, of 8 dB, at 16,968 Hz so additional controls, stimulated at MAX -55, were included. Another feature of stimulation at 12 kHz, informally observed, was that stimulation at MAX -5 for 15 minutes did not produce an appreciable shift in N_1 as it had at 3 kHz and 6 kHz.

Due to the fact that these anesthetized guinea pigs did not show complete recovery of N_1 in the typical acute experiment of 2 to 5 hours, additional groups of guinea pigs were exposed and recordings were made at selected longer times after exposure. The results of such an exposure at 6 kHz (15 min. at 104 dB SPL) with recovery of N_1 at 8484 Hz is shown in Figure 23. The main feature of the recovery is that it occurs within 24 hours.

Experiment 4

By way of summary it can be said that experiments 1 and 3 have demonstrated that the effects of sodium salicylate and intense sound are similar. Both agents, under the above conditions, have a major effect on N_1 and little or no effect on the a.c. cochlear potential. Thus, both of these agents have a similar effect and an interaction between these two agents, if given together, might be expected.

Figure 23. The loss and recovery of N_1 after intense stimulation with a 6 kHz tone for 15 minutes. The values of N_1 and the cochlear potential are shown relative to controls at 8484 Hz. These data are from three groups of animals. The data points at 0 and 1 hour post-exposure are from four animals serving as their own controls and were stimulated at MAX -5. The data points at 9 and 24 hours are from four animals exposed to 104 dB SPL compared with the mean value from four animals exposed to 74 dB SPL.


dB change in sound intensity re controls
+40
+30
+20
+10
0
-10
0
3
6
9
12
15
18
21
24
Hours Post Exposure
N1
C.P.

Experiment 4 was a study of the interaction of sodium salicylate and intense sound under the conditions of Experiment 1 and 3. In this short term part of the experiment three groups of guinea pigs were used. Group 1 - Salicylate and Overstimulation. Sodium salicylate (500 mg/kg) was injected subcutaneously and four hours after injection the ear was exposed to a 6 kHz tone at MAX -5 for 15 minutes. Five guinea pigs were used in this group. Group 2 - Saline and Overstimulation. An equivalent volume of isotonic saline was injected and four hours after injection the ear was exposed to intense stimulation, 6 kHz at MAX -5 for 15 minutes. Three guinea pigs were used in this group. Group 3 - Salicylate and Moderate Stimulation. Sodium salicylate (500 mg/kg) was injected subcutaneously and then four hours after injection the ear was exposed to moderate stimulation, 6 kHz at MAX -30 for 15 minutes. Three guinea pigs were used in this group.

The resulting changes in N_1 and the a. c. cochlear potential in each group are presented in Table III and plotted in Figure 24. No appreciable change was found in the a. c. cochlear potential, in any group, while large changes were found in N_1. An interaction between salicylate and intense sound is suggested by the fact that the greatest losses in N_1 are seen in the Salicylate and Overstimulation group.

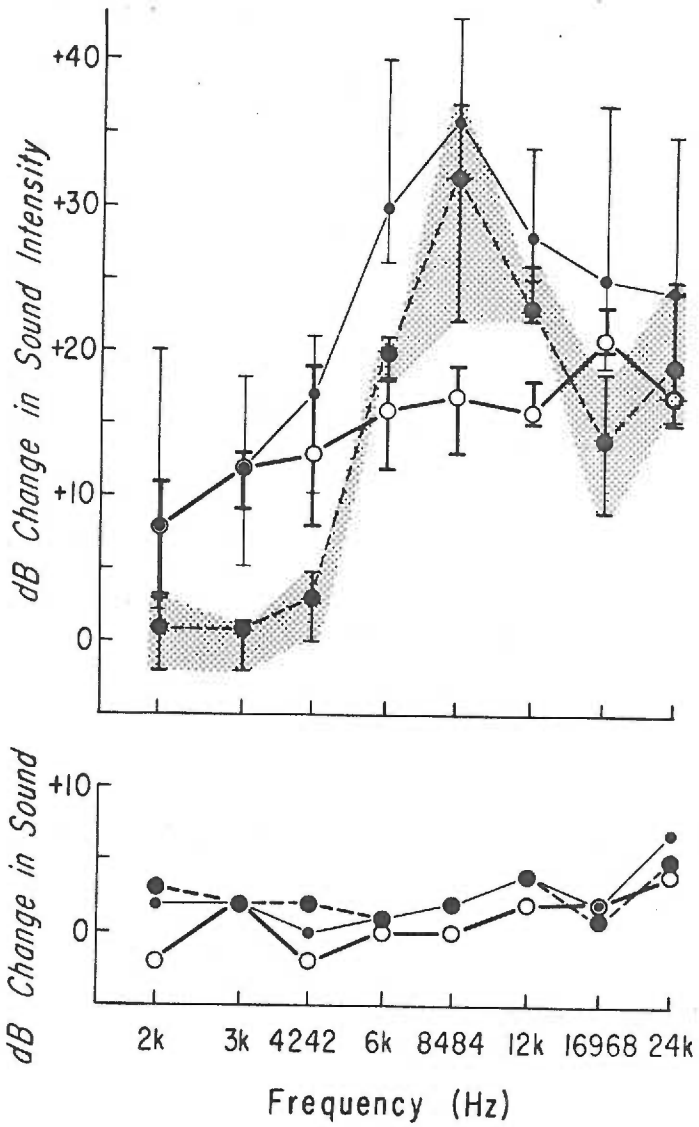
The losses in N_1 due to sodium salicylate and due to intense sound have each been shown to be temporary, see Figures 18 and 23. These agents have also been shown to interact when they are given together (see Figure 24). One would be tempted to ask, "If both agents are given together, is the effect still temporary? Or

Table III. The interaction of sodium salicylate (500 mg/kg) and intense sound. The data represent increases in the amount of sound (dB) necessary to evoke 50 μV of N_1.

Frequency (Hz)	1. Salicylate + Overstimulation					2. Saline + Overstimulation			3. Salicylate + Moderate Stimulation		
	Animal #					Animal #			Animal #		
	487-A (dB)	488-A (dB)	491-A (dB)	500-A (dB)	503-A (dB)	504-A (dB)	505-A (dB)	508-A (dB)	502-A (dB)	512-A (dB)	522-A (dB)
2k	3	12	2	20	5	3	2	1	3	11	9
3k	*	*	5	18	12	1	0	2	9	13	13
4242	19	15	10	21	21	5	0	4	8	12	19
6k	27	26	40	28	29	18	20	21	12	18	18
8484	34	32	34	43	39	37	22	37	13	19	18
12k	34	27	31	25	25	22	26	22	15	16	18
16968	37	18	27	19	22	9	19	13	23	20	20
24k	35	20	24	23	17	17	15	25	19	15	17

* Data not collected

Figure 24. The results of the short term part of Experiment 4. An interaction between sodium salicylate and intense stimulation (6 kHz at MAX -5 for 15 min.) is evidenced by the fact that the group of animals exposed to both agents shows more loss in N_1 than the other groups. The mean and range of changes, from initial recordings, in the amount of sound necessary to produce 50 μV of N_1 are shown in the upper graph. Mean changes, from initial recordings, in the amount of sound necessary to produce 1 μV of a. c. cochlear potential are shown in the lower graph.



Changes in N_1

1. Salicylate + Overstimulation
2. Saline + Overstimulation
3. Salicylate + Moderate Stimulation

Changes in the A.C. Cochlear Potential

1. Salicylate + Overstimulation
2. Saline + Overstimulation
3. Salicylate + Moderate Stimulation

does it now become permanent?" The results from the long term part of Experiment 4 are shown in Figure 25. These data suggest that the losses in N_1 from an interaction of salicylate and intense sound are not permanent.

A portion of the data collected in this experiment constituted a replication and extension of experiment 1. These data are contained in the short term portion of this experiment. Similarly to Experiment 1, electrophysiological measurements were made before and again four hours after injection in this experiment. The salicylate dosages were very similar (500 mg/kg in experiment 4 compared to 545 mg/kg in experiment 1). Experiment 4 covers a wider frequency range for N_1 measurements than was used in experiment 1. The data from this part of experiment 4 are shown in Figure 26. It is clear from this Figure that there was no change in the a. c. cochlear potential, while N_1 showed a marked decrement.

Figure 25. The sound pressure levels at 8484 Hz needed to produce 50 μV of N_1 at various times after sodium salicylate injection (500 mg/kg) and exposure to intense sound at 6 kHz. In order to observe an interaction it was necessary to wait until the salicylate effect on N_1 had developed before the ear was overstimulated. Therefore the ear was exposed to intense sound four hours after salicylate injection. The primary finding here is that the interaction of these agents, under the conditions of this experiment, apparently does not produce a permanent loss in N_1. The values shown in the first six hours post-injection represent five experimental animals and three control animals. The values at 49 hours represent four experimental animals and four control animals.

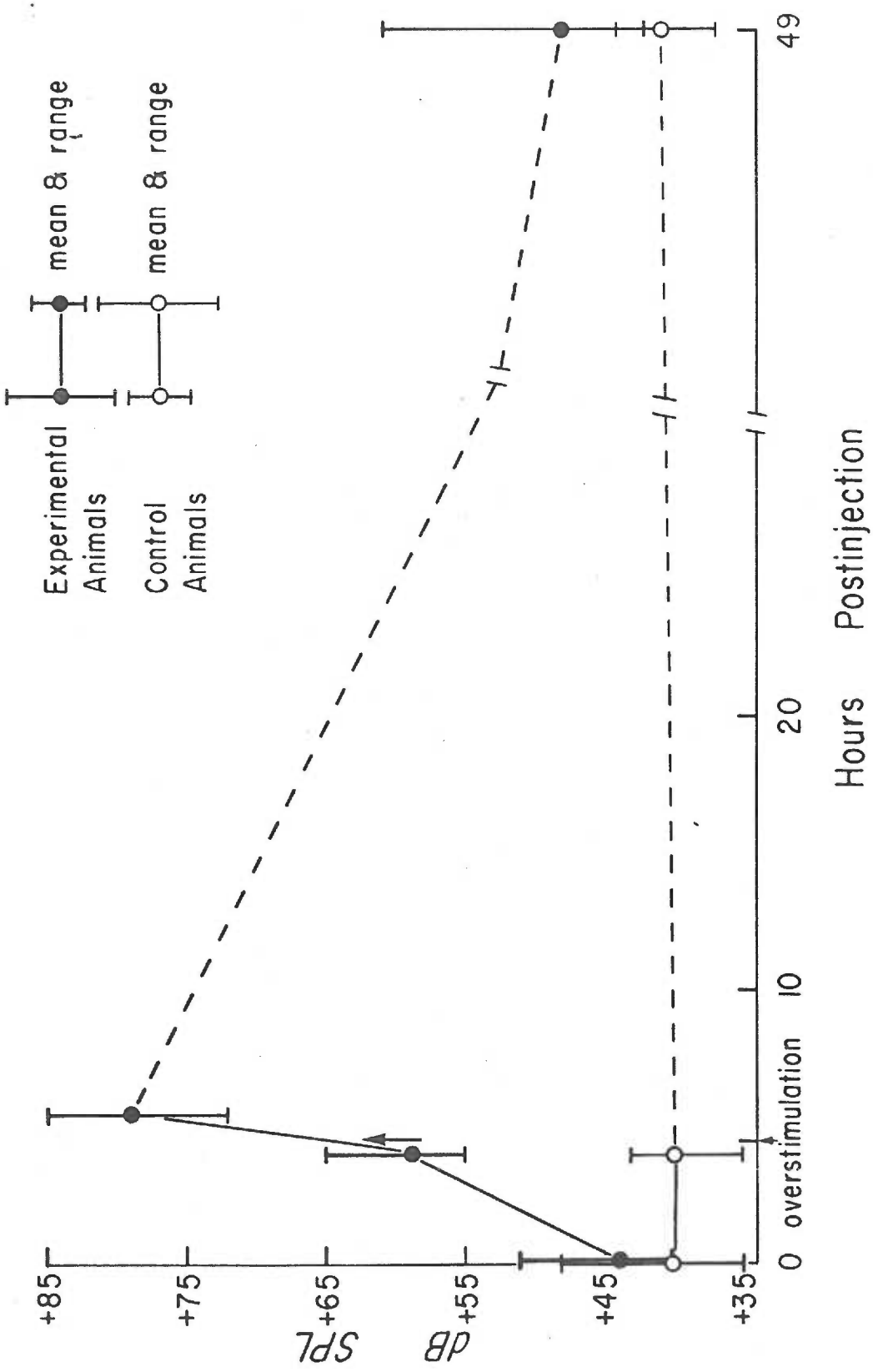
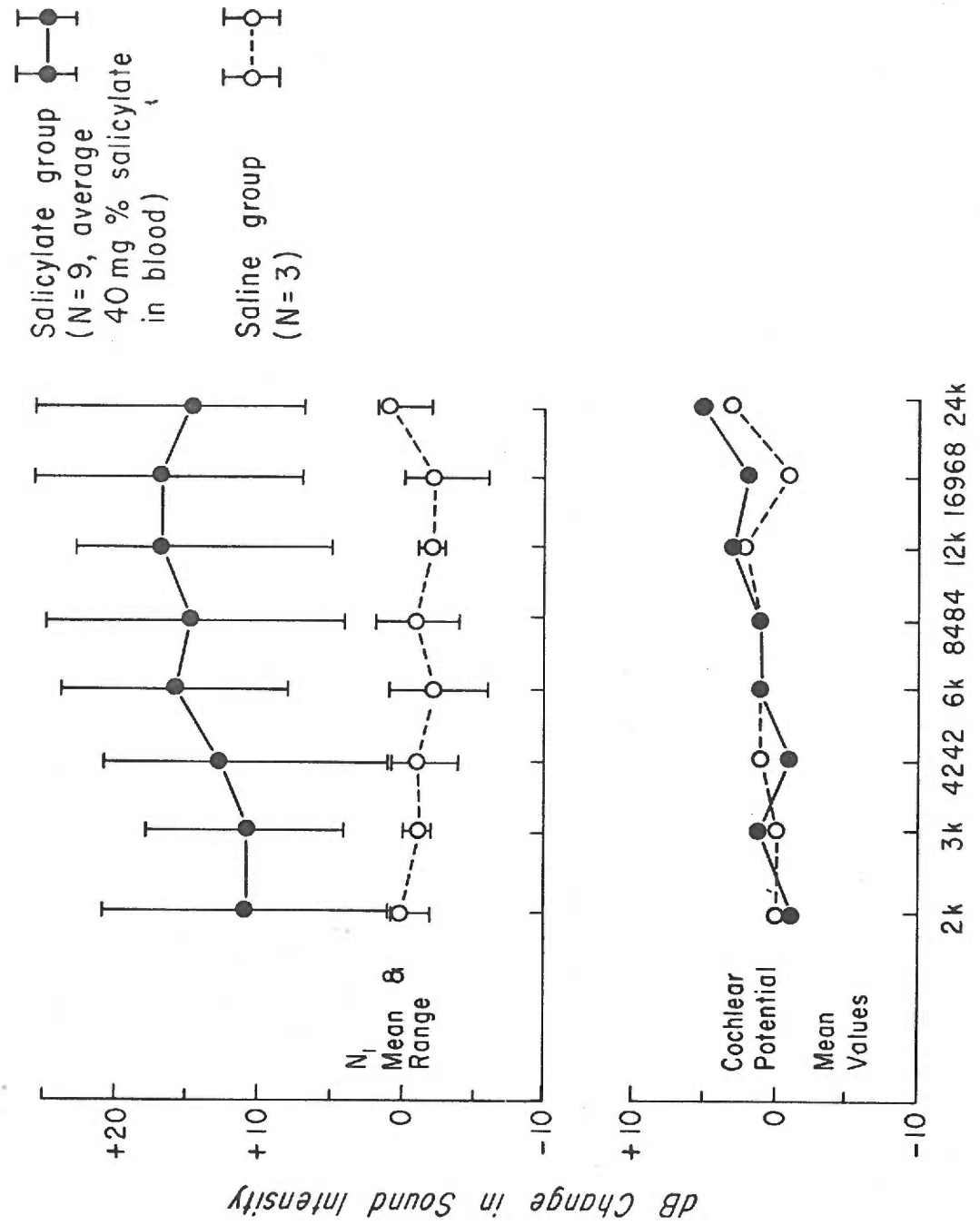

mean & range
mean & range
Experimental Animals
Control Animals
+85
+75
+65
+55
+45
+35
dB SPL
0 overstimulation
10
20
49
Hours Postinjection

Figure 26. The effects of sodium salicylate on N_1 and the cochlear potential of the guinea pig at various frequencies. These values were mean and range values obtained four hours after a subcutaneous injection of sodium salicylate (500 mg/kg) or isotonic saline (5 ml/kg). (The number of animals in the salicylate group was increased by one with the inclusion of an animal done later, but under the same conditions). It is clear from these data that there was no change in the cochlear potentials, while N_1 showed a marked decrement.


Salicylate group
(N=9, average
40 mg % salicylate
in blood)
Saline group
(N=3)
+20
+10
0
-10
N1 Mean & Range
+10
0
-10
Cochlear Potential
Mean Values
dB Change in Sound Intensity
2k 3k 4242 6k 8484 12k 16968 24k
Frequency (Hz)

DISCUSSION

Baseline data for the 1 μV a.c. cochlear potential and the 50 μV level of N_1 recorded from the round window membrane of normal guinea pigs have been presented in Figure 10 through 14. Normative data such as these have not been reported previously. In reporting these data it is thought that they may be useful for a variety of future comparisons.

The results of experiments 1 through 4 are summarized as follows. The effects of both sodium salicylate and intense sound stimulation on electrophysiological measures of cochlear function are similar. That is, both of these agents reduce the amplitude of the auditory evoked neural potential (N_1) while not affecting the a.c. cochlear potential. In each case the effect on N_1 was shown to be temporary. These findings support the hypotheses advanced concerning the effects of each of these agents.

Furthermore, an interaction between these agents was hypothesized and evidence for such an interaction was found. The interaction was also shown to be temporary.

The question of whether salicylate has an effect on the cochlear potential is an important one. Silverstein et al. (83) and McPherson (63) have reported a decrement while Wilpizeski and Tanaka (105) as well as the present study found no change and perhaps a slight increase. There are several factors which may have produced these contradictory results.

A consideration of the magnitude of the decrement in the cochlear potential is useful in order to clarify the degree of disparity between these studies. Silverstein et al. reported an average decrease of 40% in the cochlear potential evoked by a click. A 40% decrement in terms of dB would be about 5 dB. The conversion from % to dB is thought to be justified by the fact that the cochlear potential is linear over a large range (102,99) and the intensity of the click used by Silverstein et al. (83) appeared to be within this linear range. McPherson recorded changes in the 1 μV a.c. cochlear potential in the guinea pig, at frequencies from 200 Hz to 20,000 Hz, over a 5 hour post-drug administration period. Table IV is a summary of McPherson's mean data four hours after drug administration. The average decrement in the cochlear potential is 7 dB in his salicylate group and 1 dB in the control group, or a difference of 6 dB that can be attributed to the drug, choline salicylate. These findings, as mentioned above, are in contrast to the lack of change reported by Wilpizeski and Tanaka as well as the present study. There are differences between these studies which might explain the different results. These include the following: 1) The animal used. 2) Different anesthetics used. 3) Different salicylates, dosages and routes of administration used. 4) An interaction between the salicylate and the anesthetic used.

Both Silverstein et al. and McPherson used sodium pentobarbital

Frequency (Hz)	Choline Salicylate (dB change)	Saline Control (dB change)
200	-7	0
400	-8	-2
800	-8	-1
1k	-11	-2
3k	-7	-1
4k	-7	-1
6k	-9	-1
10k	-6	-2
20k	-4	-3

Table IV. The present author's summary of McPherson's (63) data of the change in the 1 μV cochlear potential four hours after drug or placebo administration.

as an anesthetic. Wilpizeski and Tanaka recorded from awake animals, while the present study used allobarbital and urethan as an anesthetic. To evaluate the possibility that the anesthetic or its interaction with salicylate produced the decrease in the cochlear potential, a cat was injected with sodium pentobarbital and sodium salicylate (300 mg/kg). This cat was not respirated in order to duplicate the method used by Silverstein et al. Recordings were made as usual. A 2 dB to 5 dB decrease was found in the cochlear potential while N_1 decreased greater than 20 dB. This decrease in the cochlear potential was not thought to be biologically significant and yet it compares favorably with the

data reported by Silverstein et al. (83). This suggests the possibility that sodium pentobarbital or its interaction with salicylate, in freely breathing cats, may have produced the decrease in the cochlear potential reported by Silverstein et al.

A visit was made to McPherson's laboratory to observe his procedure. After observing a guinea pig in which a 16 dB decrease in the cochlear potential was recorded, two hours after a 300 mg/kg dose of choline salicylate was given via an orogastric tube, the present author was allowed to use McPherson's equipment, including his sodium pentobarbital, respirator and one of his guinea pigs. An average increase of 4 dB was found in the cochlear potential two hours after a 545 mg/kg subcutaneous injection of sodium salicylate. Although the route of administration was different, this was not thought to produce the different results for the following reasons: because both Silverstein et al. and Wilpizeski and Tanaka used identical doses and routes of administration and reported opposite results; and because there are no good reasons to expect different metabolites from these different routes.

McPherson used an isotonic saline control group to compare with his experimental animals given choline salicylate. Because choline itself has pharmacologic actions, it would seem that the choline salicylate group of animals should be compared with one in which choline chloride or some other choline derivative was administered. It is suspected that McPherson's decrement in the cochlear potential may have been due to the choline portion of the

molecule, but this is only a speculation.

In the same vein, one possible weakness of the present study should be mentioned. The isotonic saline control group used in these experiments did not mimic the sodium load of the high doses of sodium salicylate that were given. The high dosage of sodium salicylate (545 mg/kg) could have produced a considerable increase in the concentration of sodium in the plasma. However, Silverstein et al. (83) reported no change in the sodium concentration in the perilymph of the cat after an injection of sodium salicylate. In addition, one would expect an increase in the amplitude of the action potentials from single nerve fibers when the extracellular concentration of sodium is increased (91). It is suggested that any future experiments control not only for the volume of injected solution but also any increased sodium load.

Falbe-Henson (22) suggested that salicylates caused an increase in intralabyrinthine pressure. This pressure was thought to dampen the movement of the basilar membrane and thus produce the hearing loss. Such a damping would be expected to decrease the cochlear potential. Thus, the findings in this study do not support the intralabyrinthine pressure hypothesis of salicylate action.

All of the studies which have recorded the auditory nerve evoked potential have found a decrement from salicylate (83,105,26). The generation of N_1 is undoubtedly dependent on hair cell activity, but precisely where the a.c. cochlear potential fits into this pattern of activity is not known at present. An excellent review

of this question has been made by Wever (99). Because the a.c. cochlear potential is affected little, if any, by salicylate, a direct effect on the hair cell is thought unlikely but cannot be ruled out. Salicylate could be acting at the synapse between the hair cell and the afferent fibers of the eighth nerve or upon the primary neurons themselves.

Salicylates could also be acting on the auditory efferent neural system. It has been shown that electrical activation of the olivocochlear tract from the brainstem produces a reduction of N_1, which has been evoked in response to sound. In addition to the reduction of N_1, a slight enhancement of the cochlear potential is also present (19,20,23,24). These same effects were found in this study, as well as in the study by Wilpizeski and Tanaka (105). Therefore,it was hypothesized that the salicylate effect was a result of stimulation or activation of efferent nerve fibers via the superior olivary nucleus in the brainstem. This was seen as a reasonable possibility because salicylates are known to stimulate other brainstem nuclei (107).

A second feature of the salicylate effect on N_1 is its apparent slow onset. As can be seen in Figures 16 and 17, the blood concentration of salicylates rises rapidly within the first hour and yet the effect on N_1 is not clearly seen for two or more hours after injection. This could be for several reasons, including slow biochemical processes and/or the slow equilibration of salicylate between the blood and the fluids of the inner ear.

The hypothesis, that the slow onset of the salicylate effect is due to the slow accumulation of salicylate in the fluids of the cochlea, was made. This hypothesis was made on the basis that the distribution of the salicylate anion between blood, cerebral spinal fluid and perilymph is largely determined by the concentration of proteins in each. The protein content affects the distribution of salicylate because 50% to 85% of the salicylate anion is bound to protein (27,53,56). For example, the entry of salicylic acid into rabbit brain from the plasma has an equilibration half-life of about 60 minutes (50). Silverstein et al. (83) measured the concentration of salicylates in plasma, cerebrospinal fluid and perilymph and his data are compatible with the equilibration/ accumulation hypothesis.

The local application of salicylates, such as by perfusing the perilymph spaces of the cochlea, is a method of raising the concentration of salicylate in the perilymph rapidly and thus shortening any lag due to slow equilibration. Local perfusion of the cochlea would also be a method of eliminating effects on brainstem nuclei. Therefore, this method was used in a attempt to test both hypotheses mentioned above.

The data obtained from animals in which the perilymph spaces of the cochlea were perfused with sodium salicylate are shown in Figure 19. While these data are from four animals, and therefore are tentative, the usual decrement in N_1 is seen after perfusion, with no appreciable effect on the cochlear potential. This effect

is reversed by a rinse with synthetic perilymph. Thus the salicylate effect does not appear to depend upon the central activation of the efferent system and the hypothesis that efferent activation produced the salicylate effect is not supported. This conclusion should be qualified, as two possibilities concerning efferent involvement remain. There is the remote possibility that a local activation of the efferent nerve fibers and terminals within the cochlea, produced the N_1 decrement. Furthermore, the salicylate effect on N_1 could be due to its action on both efferent and afferent fibers.

As mentioned earlier Figures 16 and 17 show that the rise in blood concentration precedes the salicylate effect by an hour or more. The results of changes in N_1 after perfusion of the perilymph spaces with sodium salicylate are shown in Figure 19. These decrements of N_1 were found with the first measurements taken after the salicylate perfusion. This time ranged from 2 to 14 minutes after the perfusion. Not only the decrement but also its recovery following a perilymph rinse occurred rapidly and well within 15 minutes. These results suggest support for the equilibration/accumulation hypothesis. By the same token, these results suggest that long term biochemical processes, which take more than 15 minutes, are not responsible for the salicylate effect discussed here.

It is conceivable that the nerve fibers in these cochleas were damaged when the cochlea was drilled and the pipets placed, and being damaged they are more susceptible to salicylate or in some other way different than normal. Incidentally, the five cases

presented in Figure 19 had an initial loss in N_1 which was partially recovered after the first perfusion. Therefore, the effects of the first perfusion was to increase the amplitude of N_1 to the level shown at Procedure 1 in Figure 19.

The possibility that there are additional sites of action within the auditory system is unresolved. Wilpizeski and Tanaka (105) state the problem thusly, "On the basis of eighth nerve evoked potentials, hearing losses could not be demonstrated below doses of 300 mg/kg. On the other hand, with behavioral measurements, a significant threshole shift occurs at drug levels as low as 100 mg/kg." Myers and Bernstein (69) state that, "As the salicylate level approaches 30 mg% a hearing loss between 30 and 40 dB is seen. Above 40 mg%, the hearing loss has reached a maximum at about 40 dB." The present study contained two groups of animals, shown in Table II and Figure 26, salicylate injected which may be used for comparison. These animals had blood levels of 40 to 48 mg%, on the average, and had N_1 decrements of 8 to 22 dB. Thus blood levels of 40 mg% are associated with 30 to 40 dB of hearing loss while N_1 decrements are only 10 to 20 dB. This suggests that not all of the hearing loss from salicylates can be explained by N_1 decrements and suggests further that either N_1 measures are not as sensitive as behavioral threshold measures or that there are other sites of action at higher centers in the auditory system. An experiment in which the cochlear potential, N_1 and cortical evoked potentials were recorded would perhaps clarify this question.

The character of the hearing loss in man, as mentioned earlier, is still in doubt. Whether salicylates produce an equal loss at both high and low frequencies, referred to as a flat-loss, or whether the hearing losses are greater at the high frequencies remains a question. In experiment 1 where middle and high frequency tone pulses were used to evoke N_1 there was a suggestion that the N_1 decrement was slightly greater at the high frequency. The observations in experiment 1 were confounded by an order effect, that is, the middle frequency was always collected before the high frequency. Therefore in experiment 4, where N_1 was measured from 2 kHz to 24 kHz, this order effect was eliminated. The results are shown in Figure 26. These data suggest only a slightly greater loss at the high frequencies than at 2 kHz or 3 kHz. These data are limited by the fact that frequencies below 2 kHz were not measured, for technical reasons outlined in the introduction. The resolution of this question is probably best attained by further human studies.

The results of experiment 3 and 4 in which animals were exposed to intense sound support the statements by Hawkins and Kniazuk (40) and Rosenblith et al. (79) that sound stimulation can depress the evoked potential of the auditory nerve while leaving the a.c. cochlear potential unaffected. These observations may be questioned on the basis that in all of these studies the recording electrodes were on or near the round window of the cochlea. Dallos (11) as well as others have argued that a round window electrode records activity primarily from the basal turn of the cochlea. Therefore,

it is possible that the cochlear potential in the more apical turns of the cochlea could have been affected but due to the electrode placement the reduction was not obvious. The possibility seems remote for the animals which were overstimulated at 12 kHz as frequencies above 10 kHz are thought to be represented in the basal turn. However, for the animals which were overstimulated at 3 kHz and 6 kHz this possibility should be considered.

To test this possibility, an animal was prepared with electrodes on both the apex and the round window of the cochlea and overstimulated with 3 kHz at MAX -5 for 15 minutes, the same as the animals shown in Figure 20. The usual half-octave shift was found in N_1 while the cochlear potential, measured from the apex as well as from the round window membrane, showed no appreciable change. Thus, the conclusions of Hawkins and Kniazuk as well as Rosenblith et al. were again supported.

The primary finding of experiment 3 is that when the cochlea is subjected to intense stimulation by a pure tone the resulting depression of N_1 is frequency specific. That is, N_1 may be depressed at some frequencies and not at others.

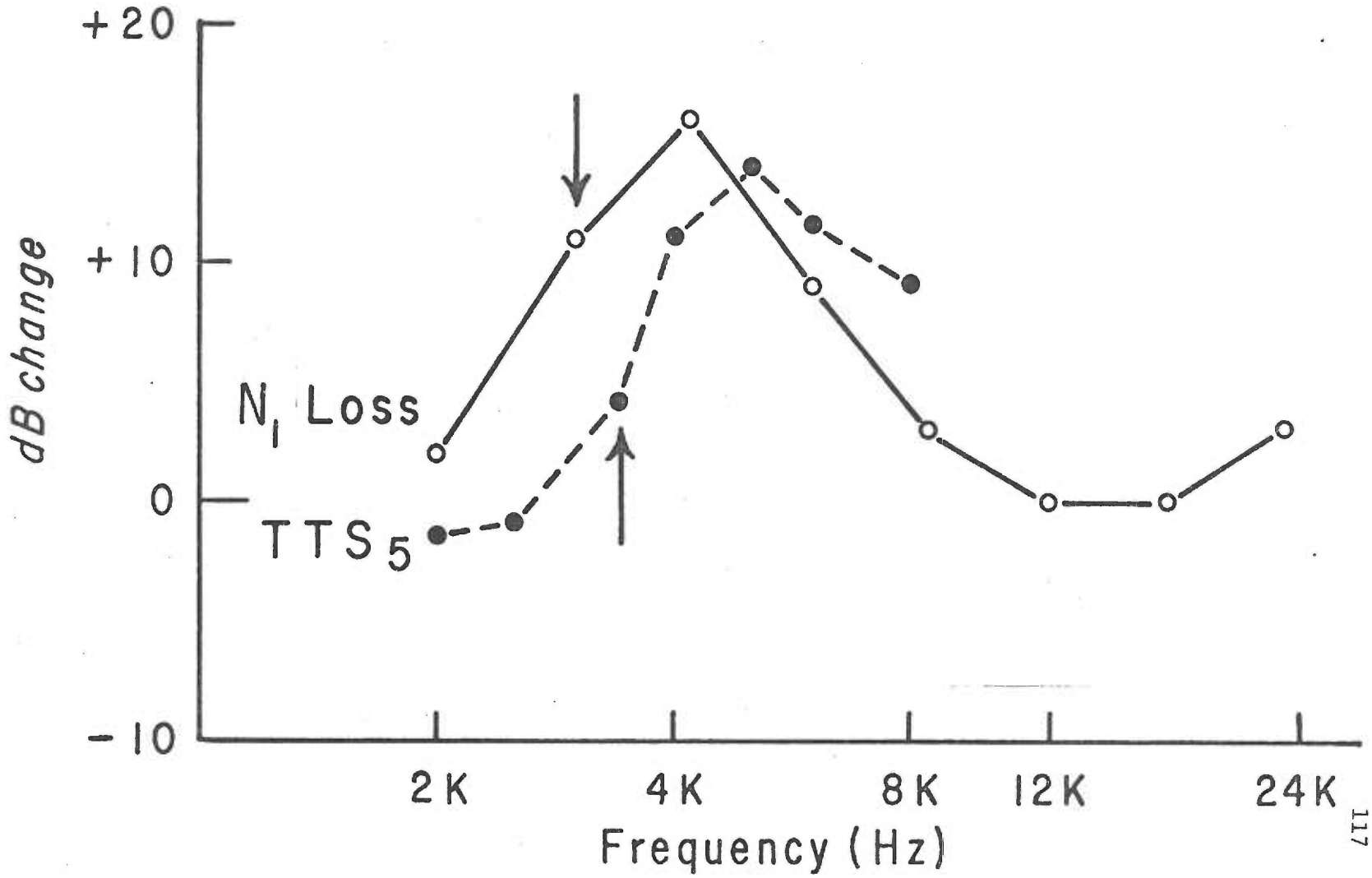
Furthermore, the frequency specificity of the N_1 depression in the guinea pig has similarities with audiologic findings in humans. A correlation between guinea pig electrophysiology and human hearing must necessarily be viewed with skepticism because in addition to species differences, the measures are entirely different. In the guinea pig the measure, in this case, is restricted to the receptor organ and in the human the measure is of hearing.

However, if the sound exposure is equated for frequency, intensity and duration a heuristic comparison can be made. Therefore, Figure 27 presents a comparison between temporary human hearing losses and N_1 decrements found in experiment 3. The general correspondence between these data is worthy of note.

The N_1 loss from intense stimulation at 3 kHz, 6 kHz and 12 kHz has been shown in each case to be maximum at half an octave above the exposure frequency. And, frequencies below the exposure frequency are not appreciably affected. These observations, as well as the fact that these N_1 decrements are reversible, are all points of similarity with human audiologic findings. It is suggested that changes in N_1 sensitivity are an important factor underlying temporary threshold shifts.

The effects of salicylate administration as well as exposure to intense sound in the present study are not thought to produce any permanent histological or cytological changes (88,55,7,69). In the case of exposure to intense sound metabolic changes are implicated (34,35,36,37,88) and activation of the efferents (88) remains a possibility. Hawkins has described vasoconstriction (or more correctly occlusion) in the vessels of the cochlea due to both noise exposure (39) and salicylate administration (38). From these observations he has suggested that anoxia within the cochlea produces the hearing loss from these agents. While the results of the present study are compatible with this anoxia hypothesis, they are also compatible with a number of other hypotheses. The data do not provide support for any single hypothesis concerning the

Figure 27. A comparison of the temporary threshold shifts (TTS) found in humans and N_1 losses found in guinea pigs are shown. The TTS data is from a study by Ward (98) and the N_1 losses were measured in the present study. Ward exposed twelve humans to a 3400 Hz tone for 5 minutes at 110 dB SPL and measured the TTS five mniutes after exposure. Average data from his study are plotted. Mean changes from pre-exposure in N_1 from four guinea pigs are shown after an exposure at 3000 Hz for 15 min. at 108 dB SPL. These N_1 losses were measured 2 to 39 minutes after exposure.


+20
+10
0
-10
dB change
N1 Loss
TTS5
2K
4K
8K
12K
24K
Frequency (Hz)

mechanism of action.

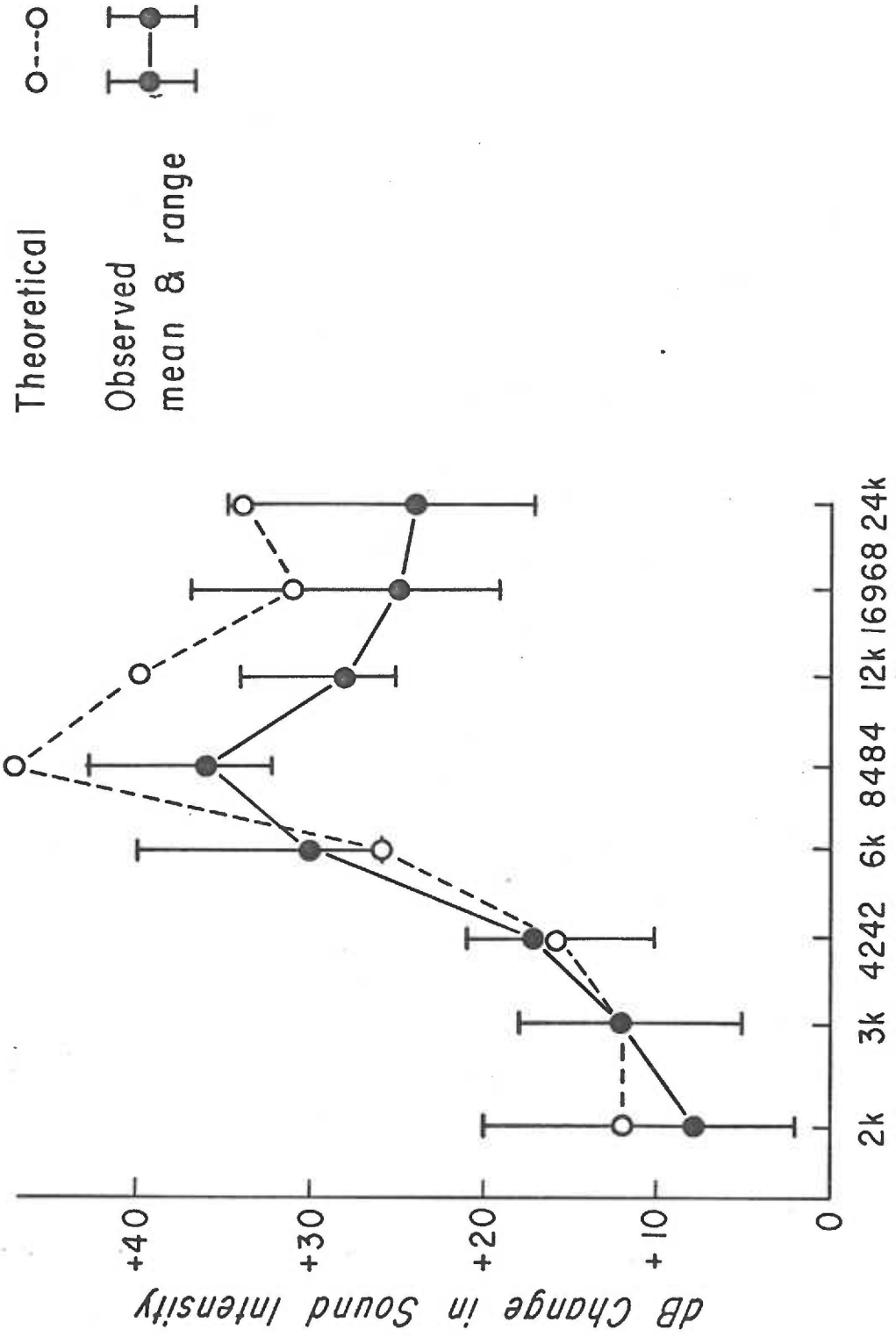
Interactions between various agents which affect the auditory system are of some interest. Since the effects of salicylates and intense sound stimulation on the cochlea are similar, one could ask the question, "Would an animal having threshold shifts due to salicylates be more vulnerable to intense sound? Or conversely, would salicylate reduce the effects of intense sound?" An affirmative answer to the latter question has been suggested in the data reported by McCabe and Dey (61). They exposed human volunteers to an intense 2 kHz tone before, during and after aspirin ingestion. The resultant threshold shifts at 3 kHz and their recovery were plotted. Their data, averaged for 5 subjects, suggests that exposure during salicylate ingestion produced smaller temporary threshold shifts (17 dB) than those prior to salicylate ingestion (27 dB). However, the losses acquired during salicylate ingestion appeared to recover more slowly.

To date, only three other studies have reported an interaction between ototoxic drugs and noise. Darrouzet and Sobrinho (12) reported histological damage in groups of guinea pigs given kanamycin alone and kanamycin followed by acoustic stimulation. The histological damage between these groups was not different and it appears that the dosage regimen of kanamycin alone caused such massive damage to the hair cells that any interaction was obscured. Another study by Dayal, Kokshanian and Mitchell (16) reported a slight loss of hair cells from kanamycin and noise exposure; all losses

were slight and the evidence for an interaction was not compelling. Jauhiainen, Kohonen and Jauhiainen (46) reported an interaction between noise and neomycin. A summary of their findings is as follows: Neomycin alone group; cochlear potential, 8 dB loss and hair cell loss 8%. Noise alone group; cochlear potential, 17 dB loss and hair cell loss 15%. Neomycin and noise group; cochlear potential, 30 dB loss and hair cell loss 80%. Thus, it seems that the interaction of neomycin and noise appears to be extremely damaging to the ear.

Experiment 4 was designed to determine whether there was an interaction of salicylates and intense sound stimulation. The results of this experiment are shown in Figure 24. An interaction is apparent but any protective or potentiating effects of salicylate are not evident. The question become, "Is the interaction greater than one would expect by a simple combination of the damaging agents?" If N_1 decrements from appropriate groups of animals are added together, one can obtain a theoretical interaction by a simple summation. Figure 28 compares such a theoretical interaction with the observed interaction shown in Figure 24. A theoretical interaction such as this should be viewed with suspicion but it appears that the observed interaction of these agents is less than what one might expect by a simple addition of damaging agents. The N_1 decrements from an interaction of salicylate and intense sound are shown in Figure 25 at six hours after injection. These N_1 decrements are shown by the data plotted at 49 hours in this figure.

Figure 28. A comparison of a theoretical interaction of intense sound at 6 kHz and sodium salicylate with an observed interaction of these agents. The interaction of sodium salicylate and intense sound produces no greater change in N_1 than would be expected by the simple addition of each agent alone. The theoretical interaction was calculated by adding the mean values of the Saline and Over-stimulation group shown in Figure 24 to the salicylate group in Figure 26. The observed value (mean and range) from Figure 24 are shown.

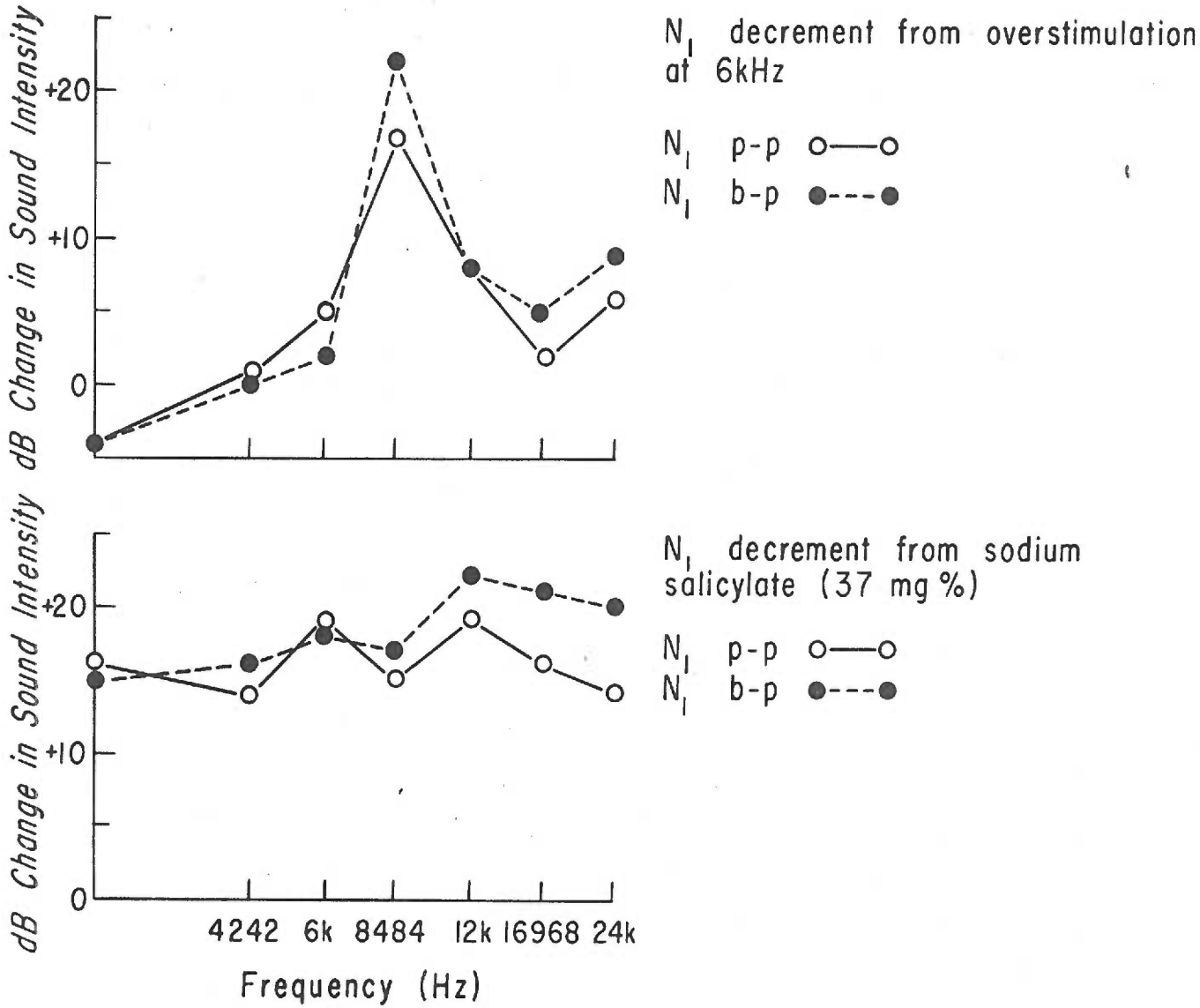

Theoretical
Observed
mean & range
dB Change in Sound Intensity
+40
+30
+20
+10
0
2k
3k
4242
6k
8484
12k
16968
24k
Frequency (Hz)

Technical Note

The data presented in this study are based on a peak-to-peak (p-p) measure of N_1 amplitude. Recently two studies have reported data which cast some doubt on the use of a p-p measure of N_1. Wang (97) and Pugh, Anderson, Burgio and Horwitz (76) have shown that N_2 can affect the p-p amplitude of N_1. For this reason Wang chose to use baseline to peak measures of N_1. He realized that a base-to-peak measure of N_1 amplitude would contain the cochlear summating potential (99) as a contaminant but apparently felt its presence was tolerable.

In order to estimate the contamination from N_2 present in the current study a guinea pig was prepared as usual and the procedure of experiment 4 was followed. Both peak-to-peak and baseline-to-peak measures of N_1 were recorded. These comparisons are presented in Figure 29. One can easily see that there are no great differences between these two measures under the conditions of this experiment.

Figure 29. The loss in N_1 from sodium salicylate and intense stimulation (6 kHz for 15 min. at MAX -5) utilizing two different measures of N_1 amplitude, peak-to-peak (p-p) and baseline-to-peak (b-p). These data are from one animal.


dB Change in Sound Intensity
+20
+10
0
N1 decrement from overstimulation at 6kHz
N1 p-p
N1 b-p
dB Change in Sound Intensity
+20
+10
0
N1 decrement from sodium salicylate (37 mg %)
N1 p-p
N1 b-p
4242 6k 8484 12k 16968 24k
Frequency (Hz)

SUMMARY AND CONCLUSIONS

A series of experiments designed to determine the effects of sodium salicylate and intense sound stimulation on the electrical activity of the cochlea are presented. The a. c. cochlear potential and the evoked potential of the cochlear nerve (N_1) were recorded from the round window membrane of anesthetized guinea pigs. These potentials were used as a measure of cochlear function.

The a. c. cochlear potential was recorded over the range from 100 Hz to 24 kHz. N_1, evoked by tone pulses, was recorded at various frequencies over the range from 2 kHz to 24 kHz. Baseline data for the 1 μV a. c. cochlear potential and the 50 μV level of N_1 from as many as seventy-nine animals are presented.

Changes in these measures induced by two agents, intense pure tones and sodium salicylate, were determined. Intense tones at 3 kHz, 6 kHz and 12 kHz were used to overstimulate the ears. Various intensities and durations of these stimuli were presented using a sealed sound system. The other agent, sodium salicylate, was administered by subcutaneous injection in one of three different dose levels. The resulting blood levels of salicylate were measured using Trinder's colorimetric method (92).

The research was divided into four experiments. Experiment 1 tested the hypothesis that the effect of sodium salicylate on the electrical activity of the cochlea is to produce a temporary depression of N_1 while leaving the a. c. cochlear potential unchanged. The results of experiment 1 showed these effects and thus supported this

hypothesis. Further, the findings support the idea that sodium salicylate (and hence aspirin) acts on the peripheral auditory system.

Experiment 2 utilized a cochlear perfusion technique and was designed to test two hypotheses. The data from experiment 2 are limited and thus the conclusions are tenative. The first hypothesis was that the slow onset of the effect of salicylate on N_1 was due to the slow accumulation of salicylate in the cochlear fluids. This accumulation hypothesis assumes that salicylates act directly on peripheral auditory structures. This data from experiment 2 support the accumulation hypothesis, in that direct perfusion of salicylate into the perilymph spaces of the cochlea produced a more rapid depression of N_1 than was seen when salicylates were injected subcutaneously.

The second hypothesis of experiment 2 was that the salicylate effect on N_1 was indirect and due to an activation of the auditory neural efferent system, as such an activation could mimic the salicylate effect. However, as local perfusion, which by-passes the efferent system, produced a depression of N_1 with no change in the a. c. cochlear potential, experiment 2 tends to contradict the hypothesis that activation of the auditory efferent system produced the salicylate effect on N_1.

Experiment 3 confirmed the hypothesis that there are sound pressure levels which will temporarily depress N_1 while leaving the a. c. cochlear potential unaffected. Furthermore, the experimental results demonstrated that the effects of intense tonal stimulation on N_1 are

related to the exposure frequency. The depression of N_1 at different frequencies has a pattern which corresponds to behaviorally measured hearing losses due to similar sounds. This pattern has the feature of a maximum loss, in hearing as well as in N_1, at one half octave above the exposure frequency. These findings also suggest that temporary hearing losses from intense sound are due to adaptation or fatigue of the auditory nerve.

Experiment 4 was designed to test the possibility of an interaction between sodium salicylate and intense tonal stimulation. The postulated interaction was confirmed by data which show that these agents, when given together, produced a greater change in N_1 than either agent alone, but not more than would be expected from a simple summation. Again, all electrophysiological effects were found to be temporary. Thus, the evidence presented here suggests that the interaction of intense sound and salicylates does not produce the disastrous interaction effects reported for other ototoxic drugs.

The method utilized for evoking N_1 at various frequencies is new in that tone bursts at discrete frequencies from 2 kHz to 24 kHz were used. Therefore, this method is potentially useful for the assessment of frequency-specific effects various agents may have on the cochlea. It is with this thought in mind that detailed methods are presented.

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APPENDIX I

Pharmacological Actions of Salicylates (107)

Local irritant effects
Salicylic acid irritates skin and mucosa. Keratolytic action is used to remove warts, corns & fungal infections.

Central Nervous System
Antipyresis - lowers abnormal temperatures, little effect on normal temperature. Analgesia - alleviates certain types of pain by a mechanism not yet elucidated.

Miscellaneous Neurological Effects
EEG changed by decrease in mean amplitude and an increase in the coefficient of variation of the waves. Depression, delirium, psychoses, stupor and coma. Seizures from toxic doses occur late during respiratory acidosis.

Nausea and vomiting are apparently due to a central effect with a contribution from gastric irritation.

Respiration - initially increased CO_2 production stimulates respiration, primarily an increase in depth. As salicylate gains access to the medulla it directly stimulates the respiratory center. Thus P_{CO2} falls and respiratory alkalosis ensues.

Acid-Base Balance and Electrolyte Pattern
Respiratory alkalosis is compensated for by renal excretion of bicarbonate accompanied by Na^+, K^+ and water.

Cardiovascular system - Ordinary therapeutic doses have no compelling actions.

Gastrointestinal Effects - In addition to nausea and vomiting, gastrointestinal hemorrhage and erosive gastritis may occur.

Hepatic and Renal Effects - Large doses apparently do not damage hepatic parenchyma but function may be impaired. Bile volume output increases but total cholate excretion is decreased; this choleretic effect may be due to direct action on liver cells. Urinary changes are not prominent except for inhibition of reabsorption of urate and phosphate.

Effects on Blood - White-blood-cell count is unaffected. Red-blood-cells, hematocrit and hemoglobin do not change with low dosages. Prolonged dosage may decrease hematocrit and hemoglobin concentration. Plasma fibrinogen content and sedimentation rate may increase.

Uricosuric Effect - Low doses (1-2g/day) increase plasma urate and decrease urate excretion. Intermediate doses (2 or 3g/day) do not alter urate excretion. Large doses (>5g/day) induce uricosuria and lower plasma urate levels.

Endocrine Effects - Adrenal medulla may release epinephrine. Adrenal cortex; ACTH stimulated which causes release of adrenocorticosteroids. Thyroid gland; complex effects on iodine and thyroxine.

Teratogenic effects are reported in experimental animals.

APPENDIX 2

Effects of Salicylates on Enzymes and Enzymatic Processes

Compound code:
(1) Salicylic acid, mol. wt. 138.12. 1 mM = 13.8 mg%
(2) Sodium salicylate, mol. wt. 160.11. 1 mM = 16 mg%
(3) Acetylsalicylic acid, mol. wt. 180.15. 1 mM = 18 mg%
(4) Sodium acetylsalicylate, mol. wt. 202.14. 1 mM = 20.2 mg%

Enzyme or System	Dose (compound)	Results (reference)
Oxidative phosphorylation	.01 - 50 mM (2)	Brain & liver homogenate, pyruvate substrate, stimulation followed by inhibition. (65)
	.01 - 10 mM (2)	Mitochondria fraction, succinate substrate, inhibition. (65)
	.01 - 10 mM (2)	Mitochondria fraction, B-hydroxybutyrate substrate, stimulation followed by inhibition (65)
Dehydrogenases		
Lactic	rat, oral, 100 mg/kg (4)	Urine assay, significant increase in amount excreted (77)
Alcohol	.05 - .30 mM (2) or (3)	Activity 80 to 0 % of control @ 38° C (30)
Malic	cat, intraperitoneally, 350 mg per kg (2)	No change in MDH activity in serum. Perilymph had lowered MDH activity (83)
Glutamic	.05 - .40 mM (2) or (3)	Activity 80 to 0% of control @ 38°C (30)
	.005 - .01 mM (2) or (3)	No change in activity @ 40°C. (30)
	.005 - .01 mM (2) or (3)	Activity 83 to 67% of control (30)
Muscile triosephosphate	.02 - .1 mM (2) or (3)	Activity 70 to 0% of control @ 38°C
	.002 - .005 mM (2) or (3)	Activity 65 to 50% of control @ 40-42°C (30)
UDPG	2.5 - 7.5 mM (2)	Activity 85 to 75% of control (52)
Glutamateoxalacetateaminotransferase	rat, oral, 100 mg/kg (4)	Urine assay, increase in amount of enzyme found (77)
Phosphatase		
Alkaline	rat, oral, 100 mg/kg (4)	Urine assay, increase in amount of enzyme found (77)

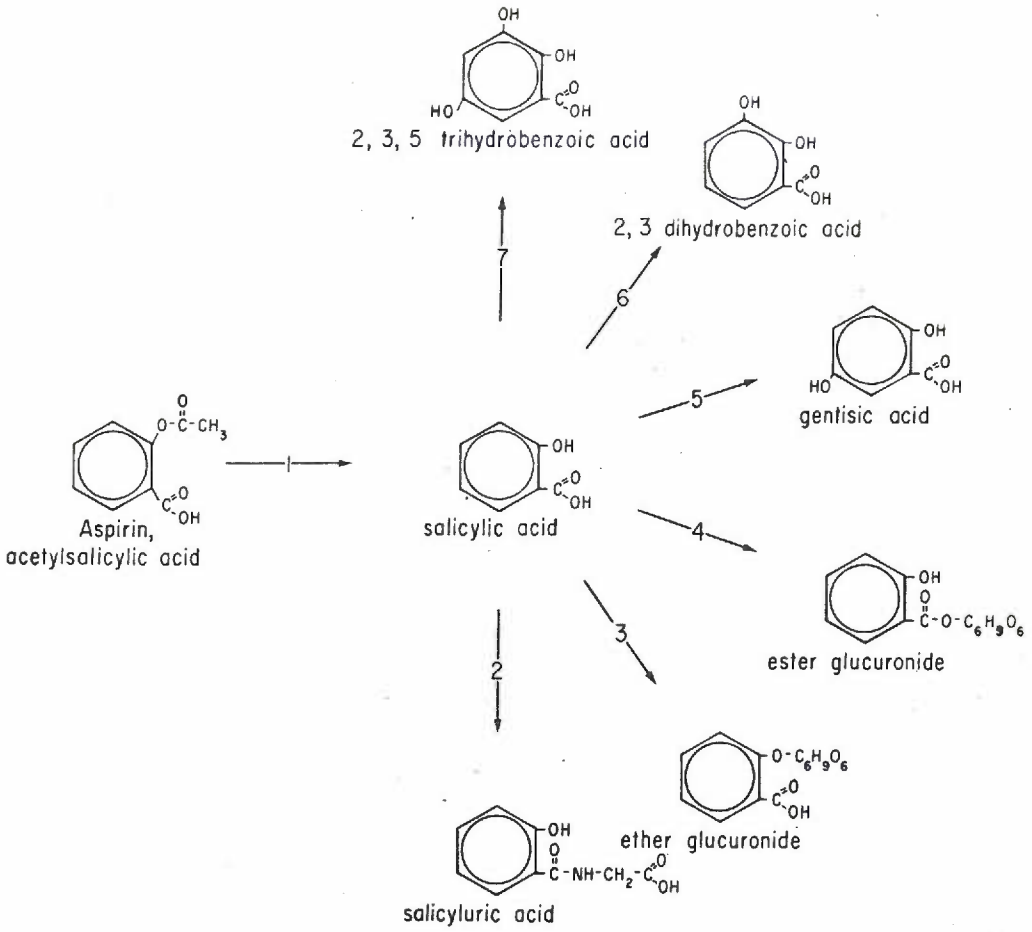
APPENDIX 3

Acetylsalicylic acid (ASA) is usually taken by oral ingestion. Hydrolysis of ASA to salicylic acid, Reaction 1 in Figure 30, takes place at a slow rate in the acid environment of the stomach, but at a much faster rate in the alkaline intestines. Salicylates are rapidly absorbed from the stomach and upper intestine and enter the circulating blood where hydrolysis continues rapidly. The liberated salicylic anion binds with blood proteins. At concentrations encountered clinically 50 to 80% of the salicylates are bound to protein (1,107). Salicylates are distributed widely throughout the body with low concentrations in the brain and skeletal muscles. The intracellular concentration is lower than the plasma concentration due to the lower pH of the cells (107).

Salicylates circulating through the liver are metabolized to salicyluric acid, Reaction No. 2 in Figure 30, ester and ether glucuronides, Reaction Nos. 3 and 4, and other metabolites, Reaction Nos. 5, 6, and 7. The concentration of these metabolites is seldom more than 7% in plasma (107). The relative amount of each of these products, called salicylates, varies with the dosage, diet, and other factors. These metabolites are found in acidic urine in the following percentages:

salicyluric acid	69%
salicylic phenolic and acyl glucuronides	20%
salicylic acid	10%
gentisic acid	1%

Figure 30. The metabolic products of acetylsalicylic acid (106). Reactions of hydrolysis and conjugation are shown. Each arrow has the reaction number on it.

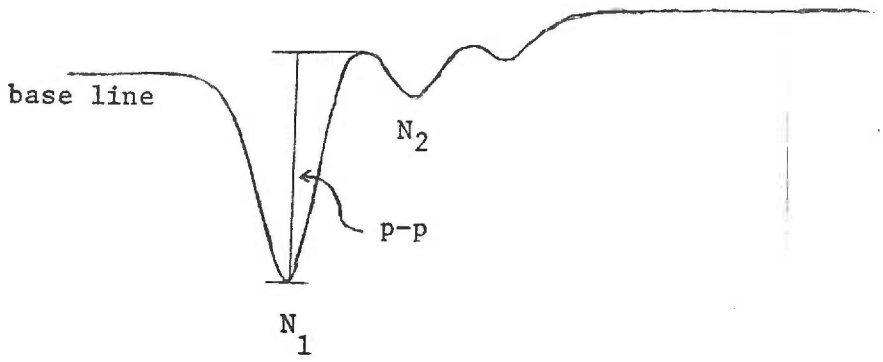

2, 3, 5 trihydrobenzoic acid
2, 3 dihydrobenzoic acid
gentisic acid
Aspirin,
acetylsalicylic acid
salicylic acid
ester glucuronide
ether glucuronide
salicyluric acid
1
2
3
4
5
6
7

The half-time of excretion of these compounds is reported to be dose dependent. In low doses 50% is eliminated in two to four hours and with high doses 50% is eliminated in 15 to 30 hours. The metabolites are similar to man in the rabbit, dog and rat (70). The metabolites of the guinea pig have not been reported but there are no obvious reasons to expect them to be markedly different from those animals in which the metabolites are known.

The dosages used in this study were chosen somewhat arbitrarily but with a few facts in mind. At the time this study began only two studies of cochlear activity were available. Silverstein et al. (83) used 350 mg/kg of sodium salicylate and found a marked effect in cats. Wilpizeski & Tanaka (105) reported that no decrements in N_1 could be found below 300 mg/kg of sodium salicylate while the reported LD_{50} for small animals was 650 mg/kg. Therefore doses of 286 mg/kg, 500 mg/kg and 545 mg/kg were used in this study. These were chosen to explore the range between the reported effective dose and the reported LD_{50}.

APPENDIX 4

In this study the peak-to-peak (p-p) amplitude of N_1, as shown below, was measured.



Four guinea pigs (# 295-A, 296-A, 298-A, and 300-A) were used to determine how many stimulus presentations should be used in each N_1 average and what repetition rate could be used without appreciably affecting the amplitude of N_1. Repeatable peak-to-peak amplitudes of N_1 were obtained when more than 8 N_1 responses were averaged. Therefore, allowing a safety factor, thirty-two stimulus presentations were averaged for each N_1 response. Several different repetition rates were used and it was found that if the stimuli were delivered more often than every 160 msec. a decrease of up to 20% was found in the p-p amplitude of N_1. If the stimuli were given every 320 msec. no appreciable decrease in amplitude was found so this repetition rate was used.

APPENDIX 5

The ingredients (in millimoles/liter) of the synthetic perilymph used in Experiment 2 were as follows:

NaCl	136.8
KCl	5.6
$CaCl_2$	2.16
$NaHCO_3$	11.9
$MgCl_2$	0.491
Na_2HPO_4	0.352
glucose	4.4

The synthetic perilymph was prepared fresh daily and the pH of the final solution was 7.95 to 8.05. This information has been reported previously by Brummett et al. (9).

When sodium salicylate was added to the synthetic perilymph, the concentration of NaCl was reduced an appropriate amount in order to keep the sodium concentration the same.

APPENDIX 6

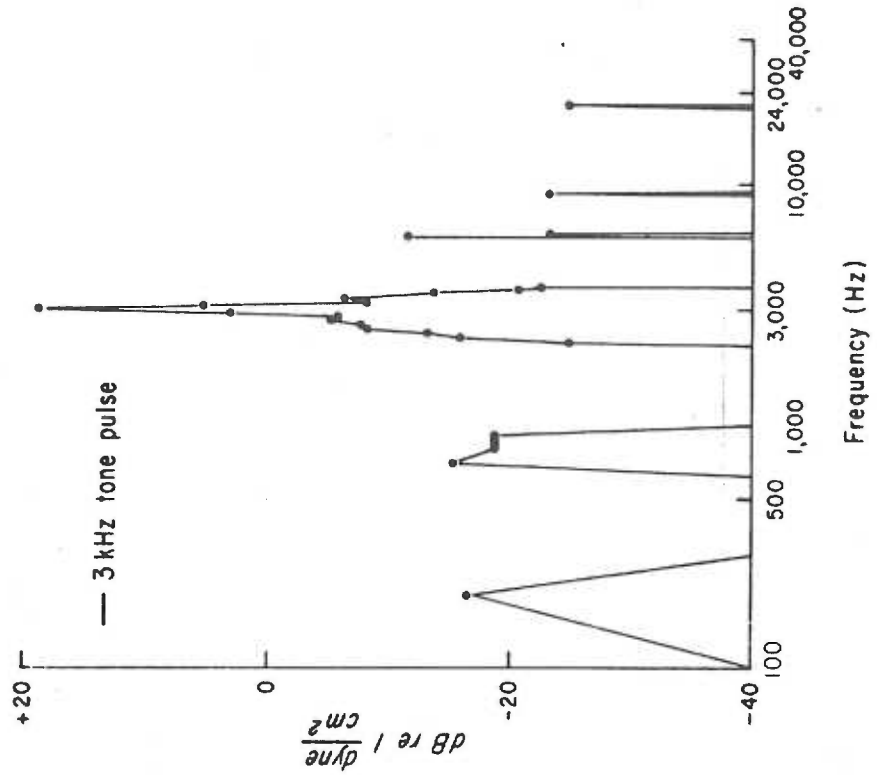
The detection and specification of distortion in acoustic stimuli is often a problem. In this study the amount of distortion which would invalidate the present N_1 method has not been determined. However, it is desirable to know what distortion products were present so that future studies could be compared with the present study. Two methods were used to estimate the acoustic distortion products in the tone pulses used to evoke N_1 in the present study.

The first method was to display the electrical signal delivered to the speaker and its acoustic output, as monitored by a B & K 1/4 inch condenser microphone, on a storage oscilloscope (97). Very little or no distortion could be seen using this method. This method of detecting distortion is only sensitive to about 5% or more. Five per cent in terms of sound pressure is equivalent to a signal down 26 dB below the fundamental. This method does not allow specification of the amount or type of distortion present, therefore another method was also employed.

The output of the calibrated microphone was monitored on the wave analyzer when trains of electrical pulses were delivered to the Beavers et al. speaker (5). The energy present at frequencies between 100 Hz and 40 kHz was measured. The energy present was measured in 100 Hz steps (using a 50 Hz bandwidth) over the range of 100 Hz to 20 kHz and in 1000 Hz steps between 20 kHz and 40 kHz.

The frequency analyses of the tone pulses used in Experiment 3 and 4, made by this method, are shown in Figures 31 thru 34. The intensities are plotted re: 1 dyne/cm^2 and a conversion to dB SPL can be made by adding 74 dB.

Figure 31. The frequency analysis of the 2 kHz and 3 kHz tone pulses used in this study.


— 3 kHz tone pulse
dB re 1 dyne/cm²
+20
0
-20
-40
100
500
1,000
3,000
10,000
24,000
40,000
Frequency (Hz)

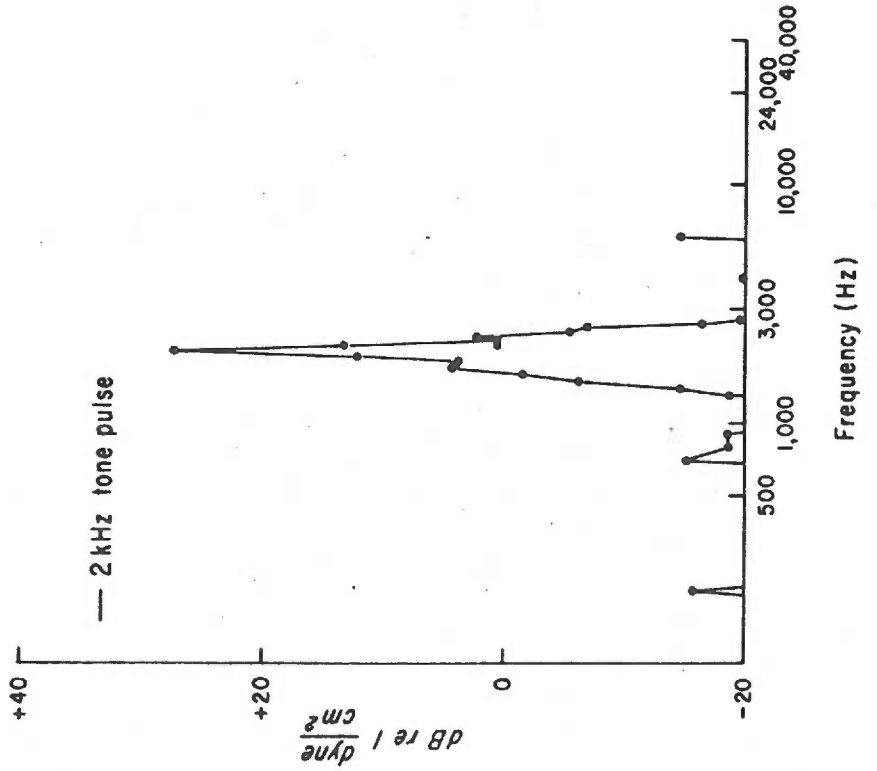
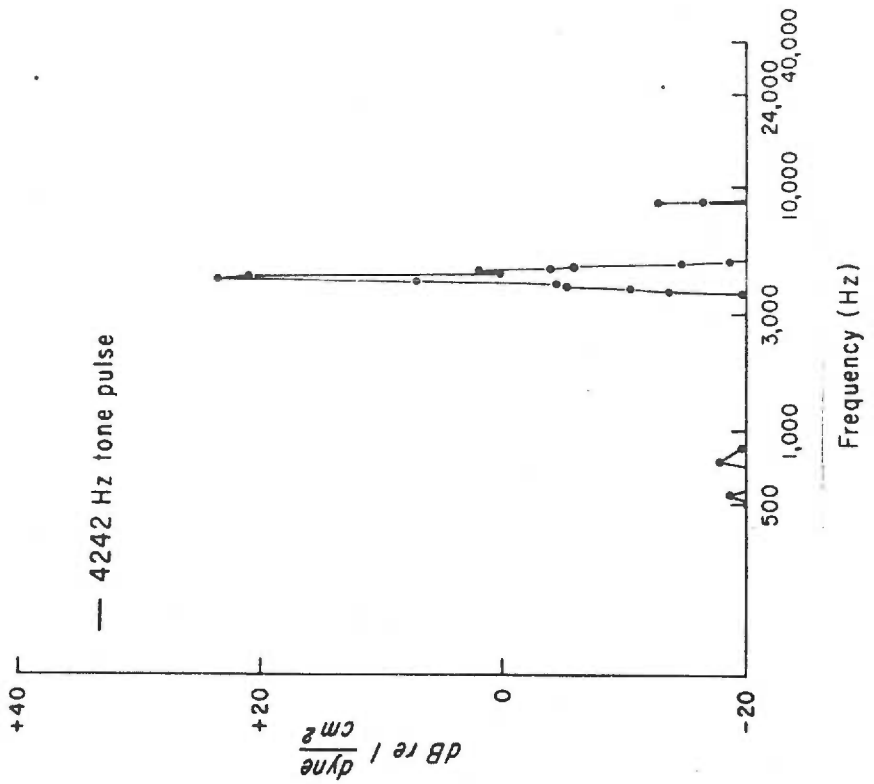

— 2 kHz tone pulse
dB re 1 dyne/cm²
+40
+20
0
-20
500
1,000
3,000
10,000
24,000
40,000
Frequency (Hz)

Figure 32. The frequency analysis of the 4242 Hz and 6 kHz tone pulses used in this study.


— 4242 Hz tone pulse
dB re 1 dyne/cm²
+40
+20
0
-20
500
1,000
3,000
10,000
24,000
40,000
Frequency (Hz)

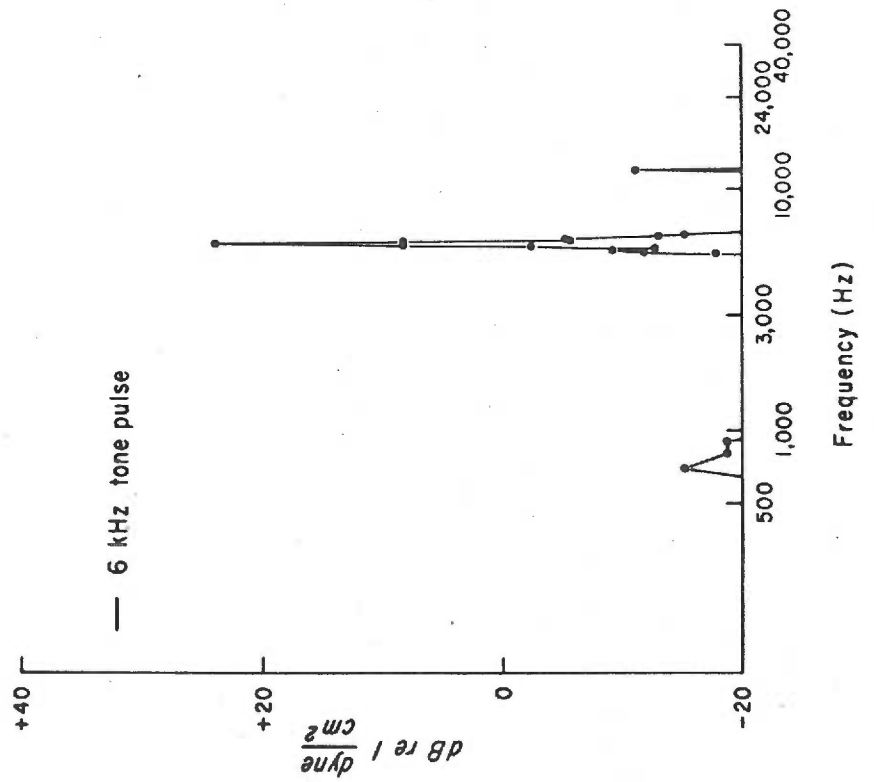
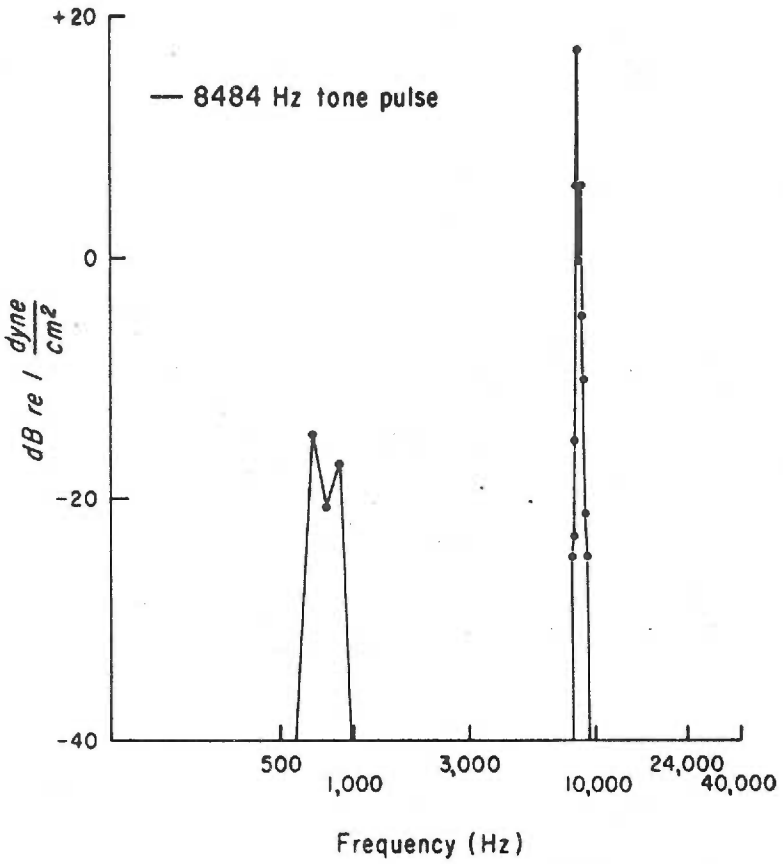

— 6 kHz tone pulse
dB re 1 dyne/cm²
+40
+20
0
-20
500
1,000
3,000
10,000
24,000
40,000
Frequency (Hz)

Figure 33. The frequency analysis of the 8484 Hz and 12 kHz tone pulses used in the present experiment.


+20
0
-20
-40
— 8484 Hz tone pulse
dB re 1 dyne/cm²
500
1,000
3,000
10,000
24,000
40,000
Frequency (Hz)

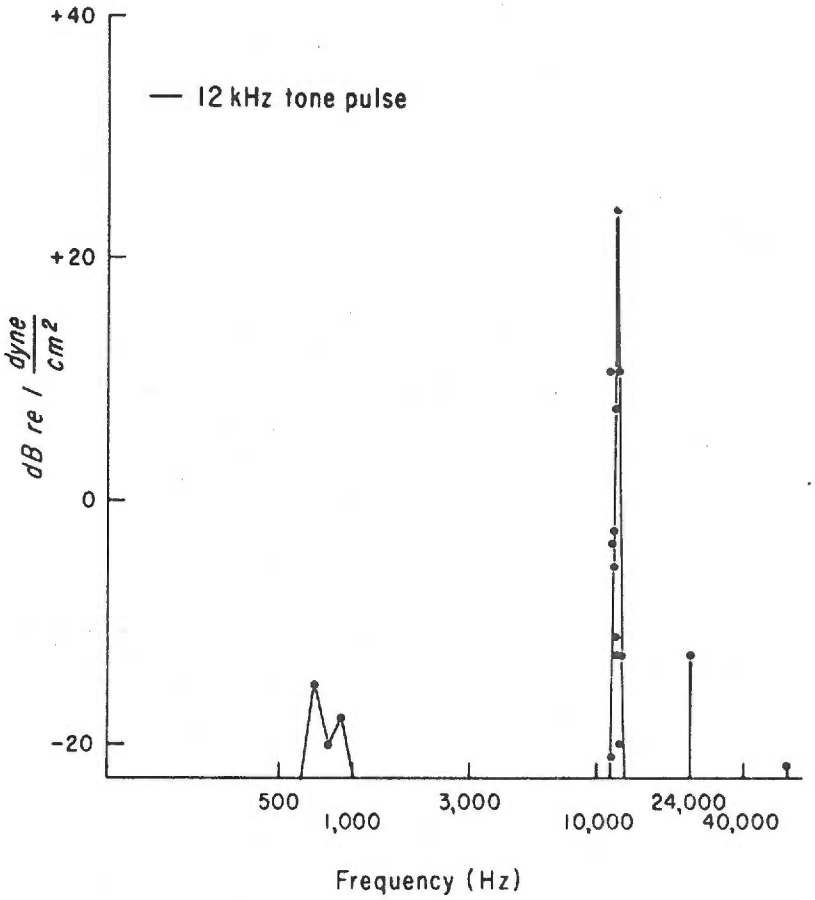
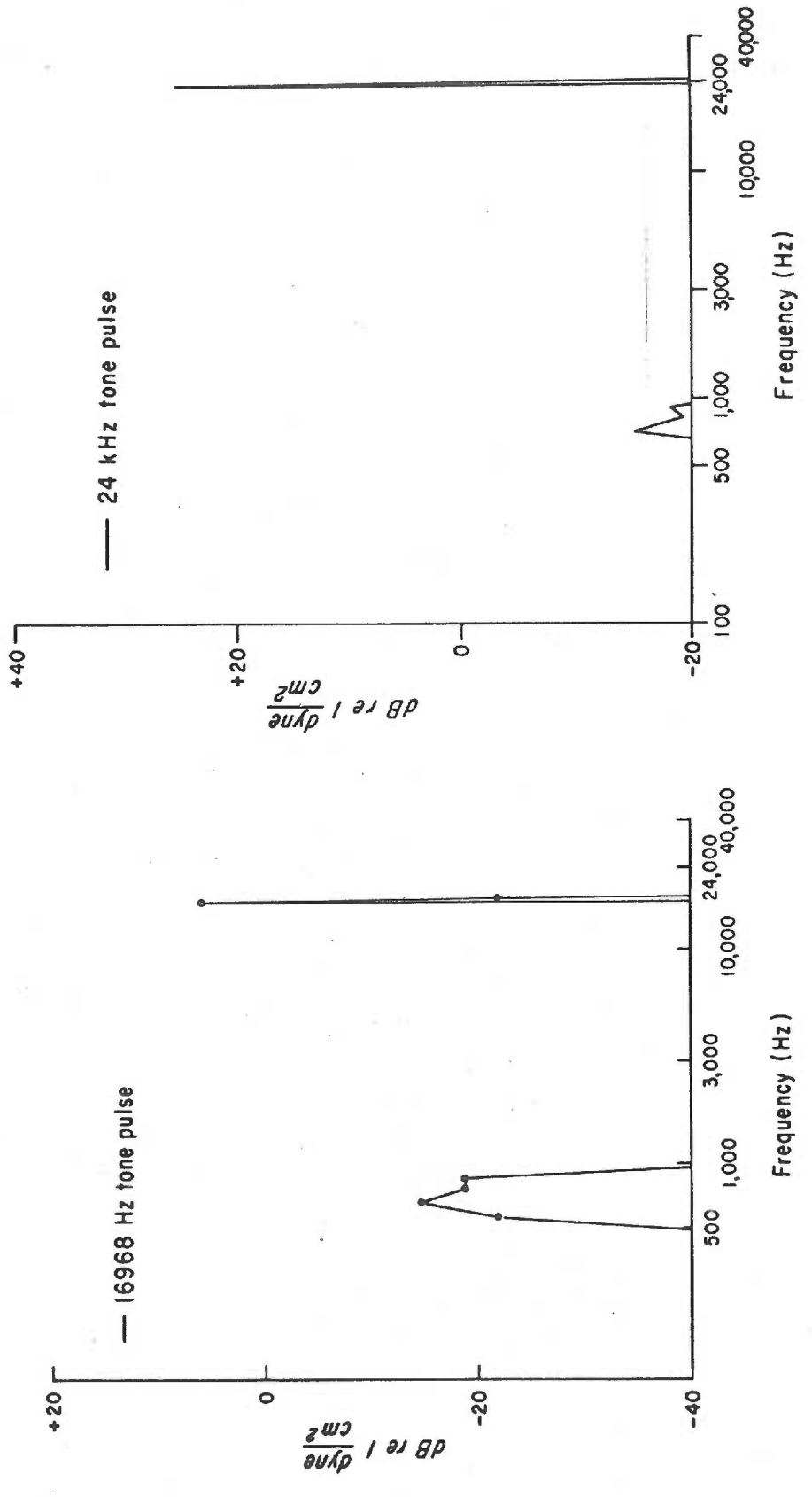

+40
+20
0
-20
— 12 kHz tone pulse
dB re 1 dyne/cm²
500
1,000
3,000
10,000
24,000
40,000
Frequency (Hz)

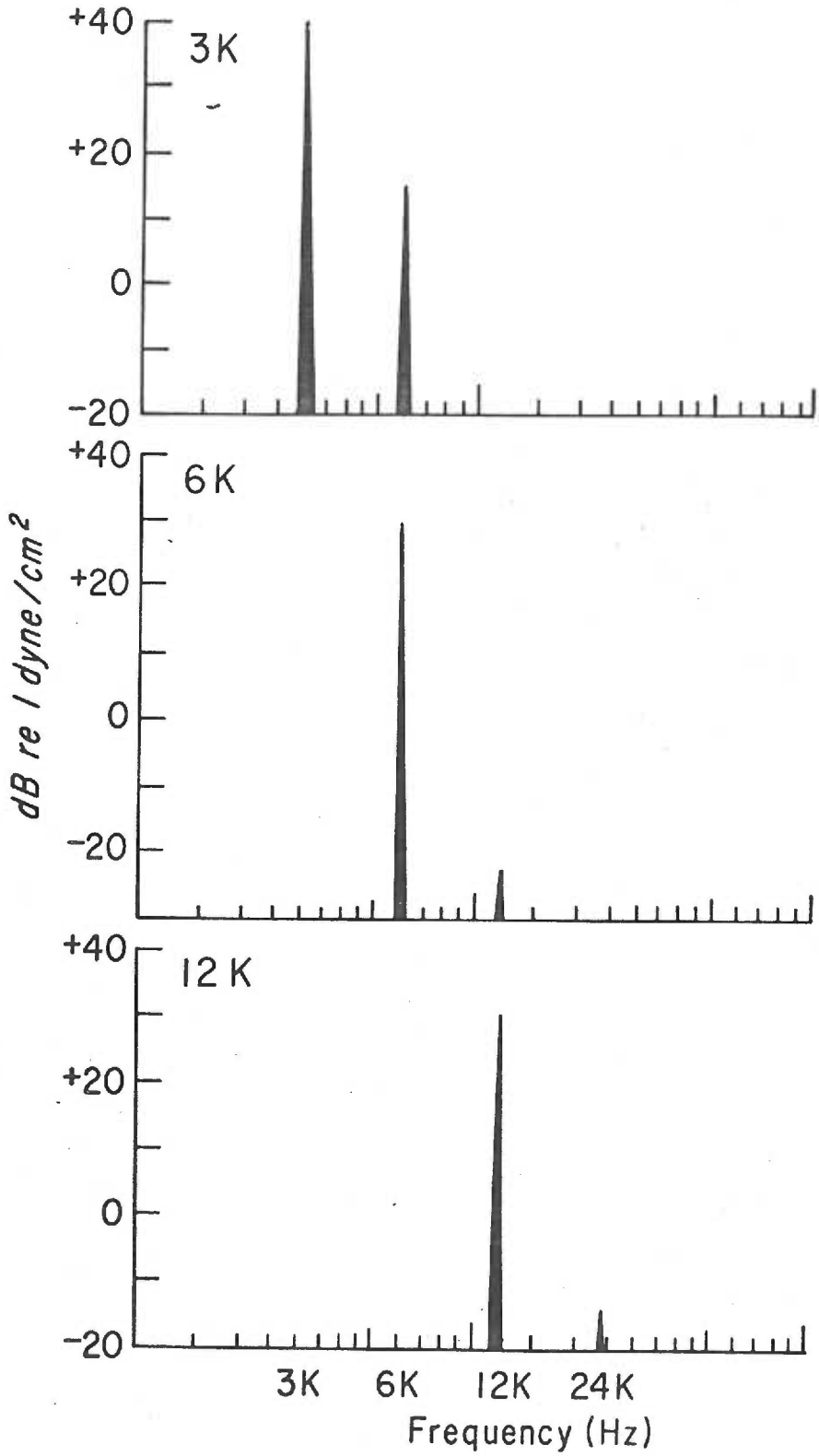
Figure 34. The frequency analysis of the 16968 Hz and 24 kHz tone pulses used in this study.


— 24 kHz tone pulse
+40
+20
0
-20
dB re 1 dyne/cm²
100
500
1,000
3,000
10,000
24,000
40,000
Frequency (Hz)
— 16968 Hz tone pulse
+20
0
-20
-40
dB re 1 dyne/cm²
500
1,000
3,000
10,000
24,000
40,000
Frequency (Hz)

APPENDIX 7

Figure 35 shows the frequency analysis of the overstimulation tones used in Experiments 3 and 4. These tones were produced by a Western Electric 555 speaker. To convert dB re 1 dyne/cm^2 to dB SPL (= re .0002 dynes/cm^2) simply add 74 dB.

Figure 35. The frequency analysis of the continuous intense tones used for overstimulation in this study. The most harmonic distortion was found in the 3 kHz tone where the second harmonic was only about 25 dB below the fundamental. The 6 kHz and 12 kHz tones had less harmonic distortion than this.


+40
3K
+20
0
-20
+40
6K
+20
0
-20
+40
12K
+20
0
-20
dB re 1 dyne/cm²
3K
6K
12K
24K
Frequency (Hz)