WADC TECHNICAL REPORT 57-647
ASTIA DOCUMENT No. AD 142078

BIOLOGICAL EFFECTS OF NOISE IN VERTEBRATE ANIMALS

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NOVEMBER 1957

AERO MEDICAL LABORATORY
CONTRACT NO. AF 33(616)-2505
PROJECT NO. 7210

WRIGHT AIR DEVELOPMENT CENTER
AIR RESEARCH AND DEVELOPMENT COMMAND
UNITED STATES AIR FORCE
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

Carpenter Litho & Prtg. Co., Springfield, O.
200 — December 1957

FOREWORD

The investigations reported herein were carried out by the Department of Zoology and Entomology and the Department of Physics of the Pennsylvania State University during the period 1 June 1954 to 30 September 1957, under Contract No. AF 33(616)-2505 with funds allotted under Project No. 7210, "The Generation, Propagation, Action and Control of Acoustic Energy." This contract was administered under the direction of the Biological Acoustics Section, Bio-Acoustics Branch, Aero Medical Laboratory, Wright Air Development Center with Captain J. E. Steele as Project Engineer.

Acknowledgments are due to the following graduate students for aid in various portions of the experimental work: L. Alboum, S. Babcock, H. Campbell, M. Clater, J. Harclerode, J. Lloyd, F. Oda, J. Parsons and H. Strickland, The authors are especially indebted to James Lloyd for help in writing the behavior action, to Fujio Oda for the material on the corona loudspeaker, to Dr. G. K. Strother for editing, and to Miss Francis Fogle who typed this report.

ABSTRACT

This report deals with the stress effects of noise on bodily functions other than hearing. It includes physiological, biochemical and behavioral effects of intense acoustic noise at low and high frequencies. Specific approaches employed are as follows: (1) flame spectrophotometric analyses of serum electrolytes, (2) serum ascorbic acid and blood sugar changes, (3) changes in adrenal and plasma cholesterol, (4) behavioral changes in noise exposed rats, mice and guinea pigs, (5) relationship of seizure-susceptibility to noise stimulation and (6) design and construction of a corona speaker for use in bioacoustic studies.

It was demonstrated that short daily exposures to intense noise of about 132-140 db pressure levels can act as a physiological stress to which rats, mice and guinea pigs can satisfactorily adapt. These studies have also helped clarify the nature of the normal physiological defense mechanisms to excessive noise stimulation. By investigating the factors determining the severity of noise as a stress stimulus and using objective measures of the limits of endurance of animals to different types of intense noise situations, one can more intelligently cope with the problem of preventing noise from becoming a serious health menace to man.

PUBLICATION REVIEW

This report has been reviewed and is approved.

FOR THE COMMANDER:

JACK BOLLERUD
Colonel, USAF (MC)
Chief, Aero Medical Laboratory
Directorate of Laboratories

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I. INTRODUCTION

Noise, an ever increasing problem in our society, has now reached the proportion in certain industrial and aircraft environments where concern should be felt regarding its role as a health menace.

For many years it has been known that above certain limits noise can cause hearing damage. These limits are now fairly accurately defined. Legislative measures have already been taken in some states to reduce this particular hazard of intense noise to humans (Nash, 1952; Symons, 1952; Nelson, 1953).

Recently, however, various indications have pointed to the possibility that noise may be harmful in ways other than through causing ear injury or hearing impairment. It is a matter of popular belief that noise not only has detrimental effects on the behavior of people (Kryter, 1952) but also may cause damage to the psychological and physiological health of individuals. In short, the belief is held by some that noise represents a harmful "stress" stimulus.

Because the term "stress" has often been ill defined and carries with it vague and subjective connotations, little attention has been focused on determining to what extent noise may act as a harmful stress. Since means are available for attacking this problem objectively, one should determine the nature of these stress effects and to what extent they may be considered as detrimental to the health of the animals, including man.

That the noise-stress problem merits serious consideration can be seen if one simply considers the large segments of our society that are exposed daily to intense noises of various sorts. Although the noise levels which have heralded our advent into an age of technology and jet power are unparalleled in human history, the noise menace is still a mere birthling. Noise experts predict a several-fold increase in noise levels in the near future if industrial developments continue at the present pace (Knudsen, 1953).

The present report deals with the biological effects of intense noise in laboratory animals. These studies are an outgrowth of experimental work begun at The Pennsylvania State University in 1947 under the direction of Dr. Harold K. Schilling. The tenure of the present studies was a three year period beginning June, 1954 through September, 1957.

Objectives:

The broad aim of the investigations included in this report was to increase our understanding of the biological effects of intense noise apart from the direct effects on the auditory apparatus. These studies mainly involved the quantitative analyses of physiological changes in tissues and organ systems which make up the "defense reservoir" of an animal to any harmful stress situation.

Specific subprojects included the following:

(1) Stress effects of noise as measured by changes in various blood constituents, such as electrolytes, eosinophils and chemicals related to hormone secretions.

(2) Stress effects of noise on the pituitary-adrenal system which represents the major homeostatic endocrine defense mechanism in vertebrate mammals.

(3) Behavioral effects of intense noise in animals.

(4) Comparison of the stress effects of intense noise at high and low frequencies.

(5) Studies of the relationship of noise to audiogenic seizures.

(6) Development of sound apparatus for use in noise studies.

II. GENERAL CONSIDERATIONS

Before dealing with the specific approaches used in the experiments, it is necessary to review certain general aspects of noise and also to define what the term "stress" signifies to the physiologist. To facilitate discussion this section is divided into the following subheadings: (1) definition of noise, (2) noise hazards, (3) concept of stress, (4) indices of measuring physiological stress and (5) justification of using animals in noise studies.

Noise:

Technically, sound is defined as a variation in pressure which is propagated in an elastic medium. In non-technical terms sound usually means pulsations transmitted as a wave in air at audible frequencies.

Noise, for the audio-frequencies, is simply defined as any undesired sound. For humans, this would fall into the audible frequency range, i.e. about 15 cycles per second (cps) to about 15 kilocycles (kc) per second. The sound pressure level, measured in decibels (db), of a sound is 20 times the logarithm to the base 10 of the ratio of the rms sound pressure (P) to the reference pressure (P_o). This is sometimes expressed

$$SPL = 20 \log P/P_o \text{ db} .$$

Originally the reference pressure was chosen as the lowest sound pressure an average person can hear at 1 kc (0.0002 dyne/cm^2 or $2 \times 10^{-4} \mu$ bar). Although this value no longer represents the minimum threshold of detectibility for humans, for convenience it is still used as the reference pressure in bioacoustics.

Two more terms which are pertinent to the present study are random and white noise. Random noise is a sound wave where instantaneous amplitudes occur, as a function of time, according to the normal (Gaussian) distribution curve. Random noise need not have a uniform frequency spectrum. White noise is a sound having a spectrum which is continuous and uniform as a function of frequency. White noise need not be random. "Several observers have shown that the use of a random noise in tests of loudspeakers is effective in smoothing out the sharp peaks and valleys introduced into the response curves in indoor measurements by the room, provided the normal modes of vibration of the room are not too widely separated" (Beranek, 1950). It is similarly desirable to smooth out these peaks and valleys when doing noise studies using animals. For all practical purposes the broad band noise stimuli used for our animal studies were both random and white for the particular frequency bands employed.

An appreciation of the significance of noise levels can be obtained from Fig. 1. This chart of common noise levels is modified after those given by Bonvallet (1948) and Knudsen (1952). Parrack (1949, 1952) reported that the 120 db level is considered harmful since temporary or permanent hearing loss can occur if this level is exceeded. More recent work on damage risk criteria for short and long time noise exposure indicates the harmful level may be considerably lower than this, i. e. approximately 90 db under some conditions (Eldred et al, 1955, Rosenblith et al, 1954).

It is generally conceded that as far as ear damage is concerned, it is the high intensities that are potentially hazardous and not the frequencies as such. This does not mean, however, that frequencies are unimportant. It is known that the sound pressure required to excite the ear below 100 cps and above 10 kc is several hundred times greater than the pressure needed for auditory perception in the 1 kc to 3 kc frequency band. It has also been shown that the frequency, as well as intensity, is a parameter of the annoyance value of sound (Kryter, 1948) and therefore probably plays an important role in the stress effects of noise in animals.

Noise Hazards:

This brings us to the problem of what constitutes a noise hazard. To industry, noise represents a problem for three main reasons: (1) noise results in interference with communication and warning sounds, thereby contributing to the cause of accidents; (2) workers are filing claims for compensation for loss of hearing. In New Jersey it is said that over five million dollars in claims have been filed against one company and possible claims in New York might approach one billion dollars, and (3) intense noise may result in ear damage or complete deafness.

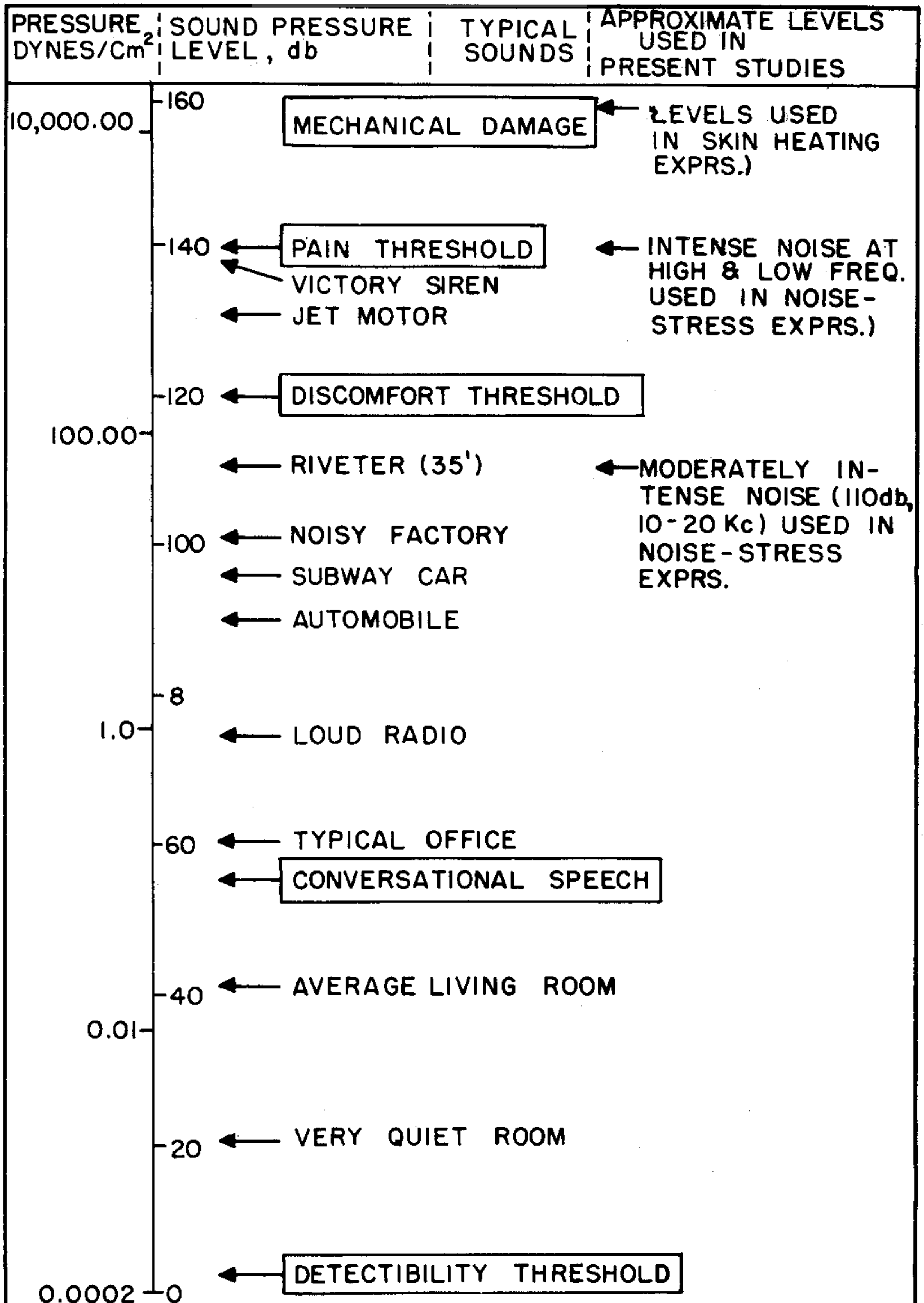


FIG.1, CHART OF SOUND LEVELS OF COMMON NOISES

Other effects of noise which have been described include the following: annoyance, nervous irritability, decreased work performance, decreased mental performance, muscular asthenia, vertigo, fatigue and decreased reproductive capacity.

Some investigators have interpreted these as possibly representing warning signs of a stress that is not yet severe enough to cause more objective manifestations (Parrack, 1949). Other workers have accumulated evidence to support the contention that intense noise exposure acts as a harmful non-specific stress. At present there is no unequivocal evidence to prove that noise can impair the psychological or physiological health of individuals. On the other hand, the contradictory evidence relating to this problem does not permit one to deny the possibility that noise under some conditions can cause physiological damage apart from hearing loss.

Concept of stress:

Before considering the approaches one can employ in studying the stress effects of noise, it is necessary to clarify what is meant by a non-specific physiological stress and under what circumstances it is harmful.

Stress has been defined by Selye (1956) as "the state manifested by a specific syndrome which consists of all the non-specifically induced changes within a biological system." In simpler terms a physiologic stress stimulus is any situation which elicits a defense response in an animal. Stress is a general term used to describe the sum total of the animals' defense response mechanism.

It has been shown that the anterior pituitary and the adrenal cortex play the cardinal roles in coordinating the defense of an animal during stress. Selye has described the role of the adrenal in the systemic response to stress under the heading "general adaptation syndrome" (G-A-S); this syndrome can be divided into three stages, the alarm reaction, the stage of resistance and the state of exhaustion. An excellent description and critical evaluation of the adaptation syndrome can be found in a recent review by Sayers (1950). For purposes of brevity, the important aspects of the G-A-S which directly bear on the studies reported herein are as follows: (1) the bodily defense reaction mediated through the pituitary adrenal pathway is not dependent on the nature of the stress stimulus, hence the name non-specific stress response: (2) the degree of adreno-cortical activation is directly proportional to the severity of the stress stimulus and (3) adrenal cortical activity is accompanied by specific changes in target organs under the influence of adrenal secretions. Figs. 2 and 3 schematically summarize the pituitary-adrenocortical relationships. In Fig. 2 a stimulus such as noise activates the anterior pituitary through the hypothalamus. The pituitary in turn elaborates adrenocorticotrophins (ACTH) which activate the adrenal cortex. Cortical hormones through their action on target organs increase the organism's resistance to the stress. The major target organs which reflect increased adrenocortical activity are summarized in Fig. 3.

It is evident then that an objective measure of the severity of a physiologic stress can be made through the analysis of adrenal changes and changes in target organs influenced by adrenal secretions. One criterion that can be used to assess the harmful nature of noise is the demonstration that an animal fails to adapt to noise stimulation, i. e. when pathological changes appear in the target organs due to excessive stimulation by the adrenal.

Indices of adrenal cortical activity:

Adrenal cortical activity can be measured in a variety of ways. Unfortunately, as with most bioassay techniques, all have certain limitations and therefore, no single measure is reliable if used alone. However, a combination of several independent measures can provide one with an accurate estimate of adrenal activity.

The major indices of adrenal activity currently used are as follows:

(1) Changes in the concentration of adrenal or plasma cholesterol and ascorbic acid. These reflect the output of pituitary ACTH. Cholesterol is a precursor of adrenocortical steroids and ascorbic acid is thought to be somehow associated with production of adrenal corticoids.

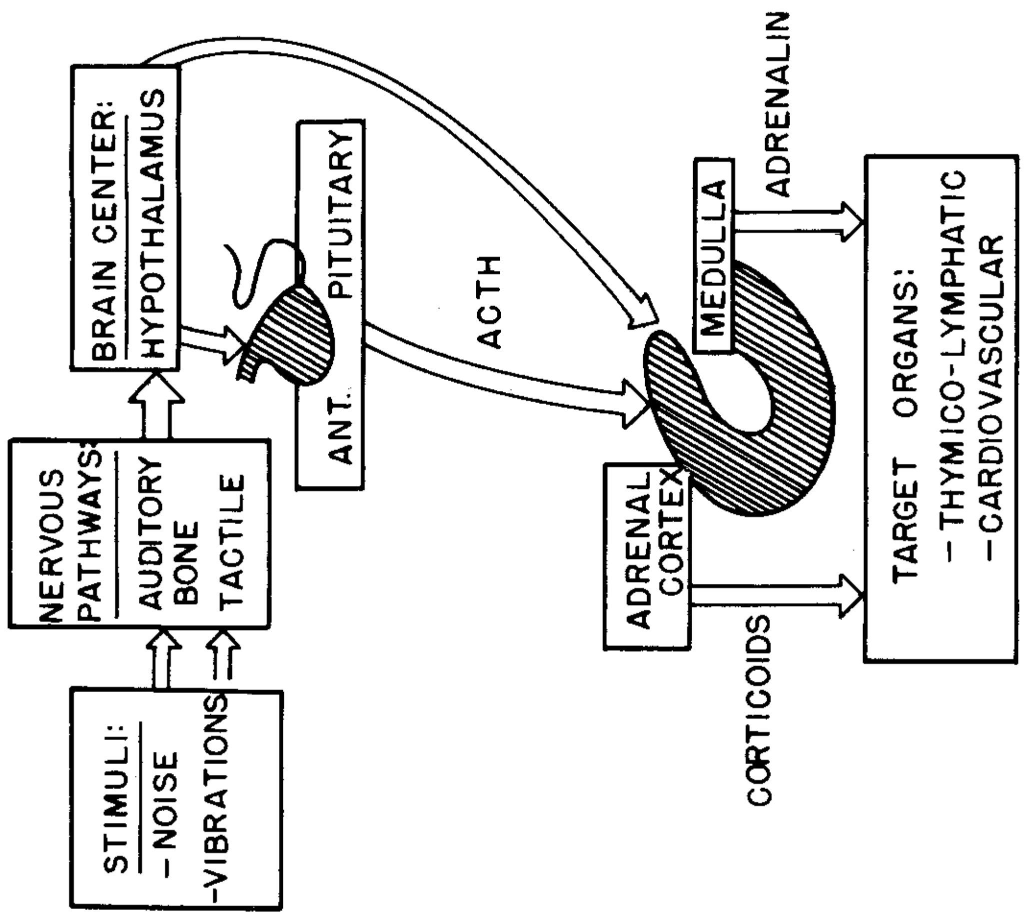


FIG. 2, SCHEMATIC DIAGRAM OF PITUITARY –ADRENAL RESPONSE TO NOISE STIMULATION

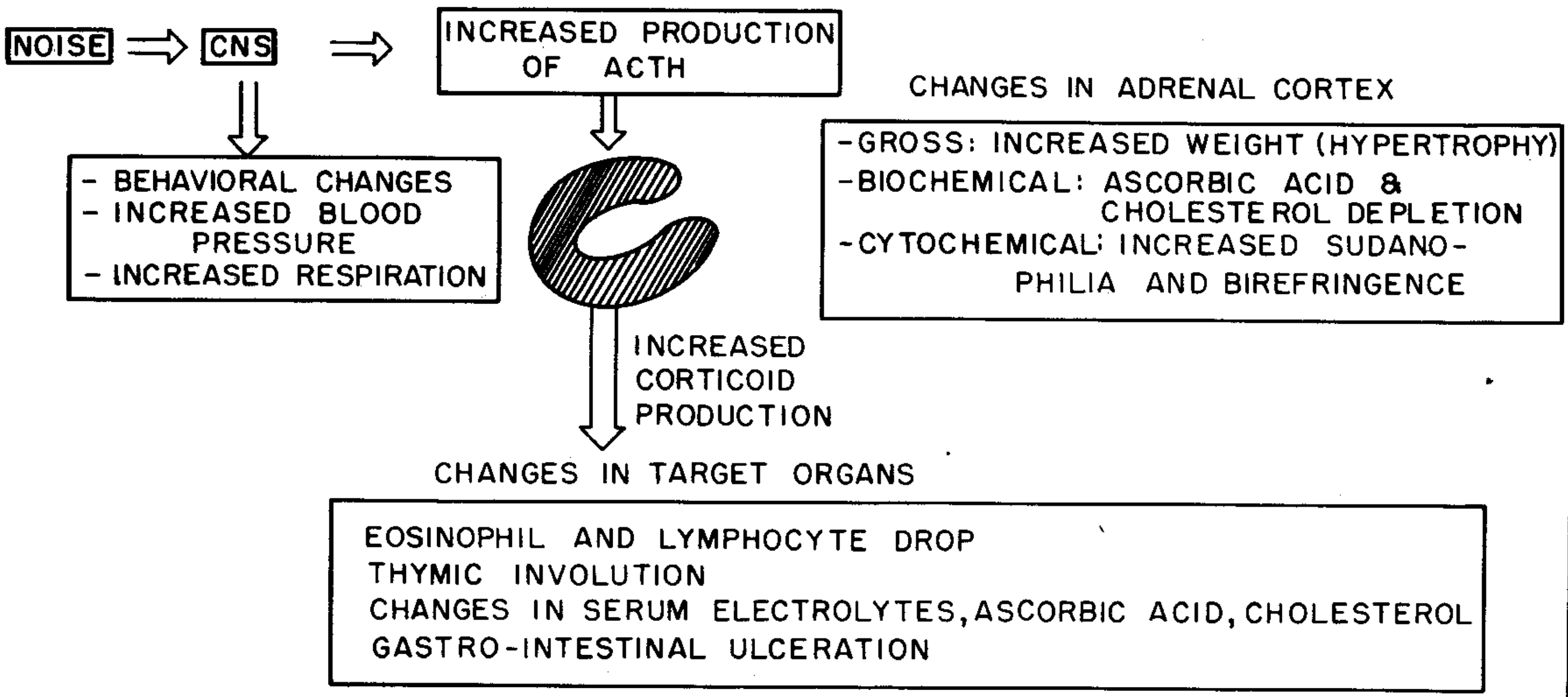


FIG. 3, SCHEMATIC SUMMARY OF SYSTEMIC CHANGES ASSOCIATED WITH INCREASED ADRENO-CORTICAL ACTIVITY.

(2) Changes in adrenal size which can be measured by taking adrenal weights or measuring changes in the width of the adrenal cortex microscopically.

(3) Histochemical changes in the adrenal. Adrenal steroids are sudanophilic and birefringent.

(4) Changes in certain blood elements, i.e. electrolytes, blood sugar and eosinophils.

(5) Changes in the target organs, especially the thymus.

All of the above criteria were employed in the present studies to evaluate the stress effects of noise in animals.

Animal experimentation:

It is appropriate at this point to clarify the reasons for using animals rather than humans for studies of noise as a stress, Simply stated, one cannot use humans because the type of information needed cannot be obtained without sacrificing animals and doing histological studies during the course of the experiment.

We can recognize our dependence on the laboratory animal for noise studies more clearly when we realize that almost all of our knowledge regarding the functional aspects of human hearing and ear injury has been obtained by extrapolation of animal data to man. Two reasons account for this. The first is that the hair cells of the organ of Corti are very susceptible to post- mortem degeneration; the second is that clinical case histories seldom contain necessary data on previous exposures of patients to noise.

As with studies of ear damage, the laboratory animal is often better suited than man as an experimental subject. In the foregoing sections it was pointed out that the adrenal cortex occupies a key position as an organ of homeostasis; among the adrenals' more important functions is its ability to endow an organism with resistance to all types of stress stimuli. Our present indices for the assessment of adreno-cortical activity require sacrificing the animal and making microscopic analyses of various organs. Fortunately the endocrine complex of laboratory animals is structurally and functionally the same as that in man. One can thus extend much of the information obtained from noise studies on animals to solve problems in man.

In summary the major reason for choosing animals rather than humans for noise studies resolves itself to the need of doing controlled experiments. Interpretation of how noise affects animals gain validity to the extent that one has information on the following: (a) previous noise history, (b) accurate description of noise stimulus, (c) time interval between exposure and autopsy, (d) analysis of tissue changes in various organs and (e) possible role of extraneous factors in causing these changes. These conditions are usually met by obtaining or rearing animals of known genetic background and studying noise-exposed and unexposed control animals under controlled laboratory conditions.

III. MATERIALS AND METHODS

Sound Apparatus:

The following is a brief description of the main types of sound generators used for research on the biological effects of sound in animals.

a) Penn State High Frequency Siren: This was originally designed and constructed by Allen and Rudnick (1947). Sound levels of about 160 db can be attained with this siren at almost any frequency from 3 to 30 kc. At some isolated frequencies acoustic pressures of 168 db can be reached. It has been used for studies on ceramic probe microphones (Ackerman and Holak, 1954), acoustic heating of haired and hairless mice (Danner et al., 1954) and for studies on skin damage to mice in intense sound fields (Frings et al., 1948, 1951; Anthony and Danner, (1955). Supplemental studies on adrenal changes following skin irradiation of mice with high intensity sound were completed during the tenure of the present contract (Anthony, 1956). These were originally begun under Contract No. AF 33(038)-786. Fig. 4 shows the apparatus used for ultrasound experiments with the air siren shown on the right and monitoring equipment on the left.

b) High frequency sound projectors: Two main types of speakers have been used as high frequency loudspeakers, a W. E. 713C receiver and a Saltshaker microphone, type AL 633A. Their principle advantages are their low power requirements and their high output in the 15-25 kc range. These were used for routine testing of mice for audiogenic-seizure susceptibility. They were also employed in studies of the stress effects of exposing mice to moderately intense high frequency noise (110 db, 10-20 kc). Fig. 5 shows the experimental set up used with the W.E. 713C speaker mounted on a reverberant exposure chamber.

c) University Model B-24 Loudspeaker System: Sound equipment was constructed for use in studies concerned with biological effects of high intensity, low frequency

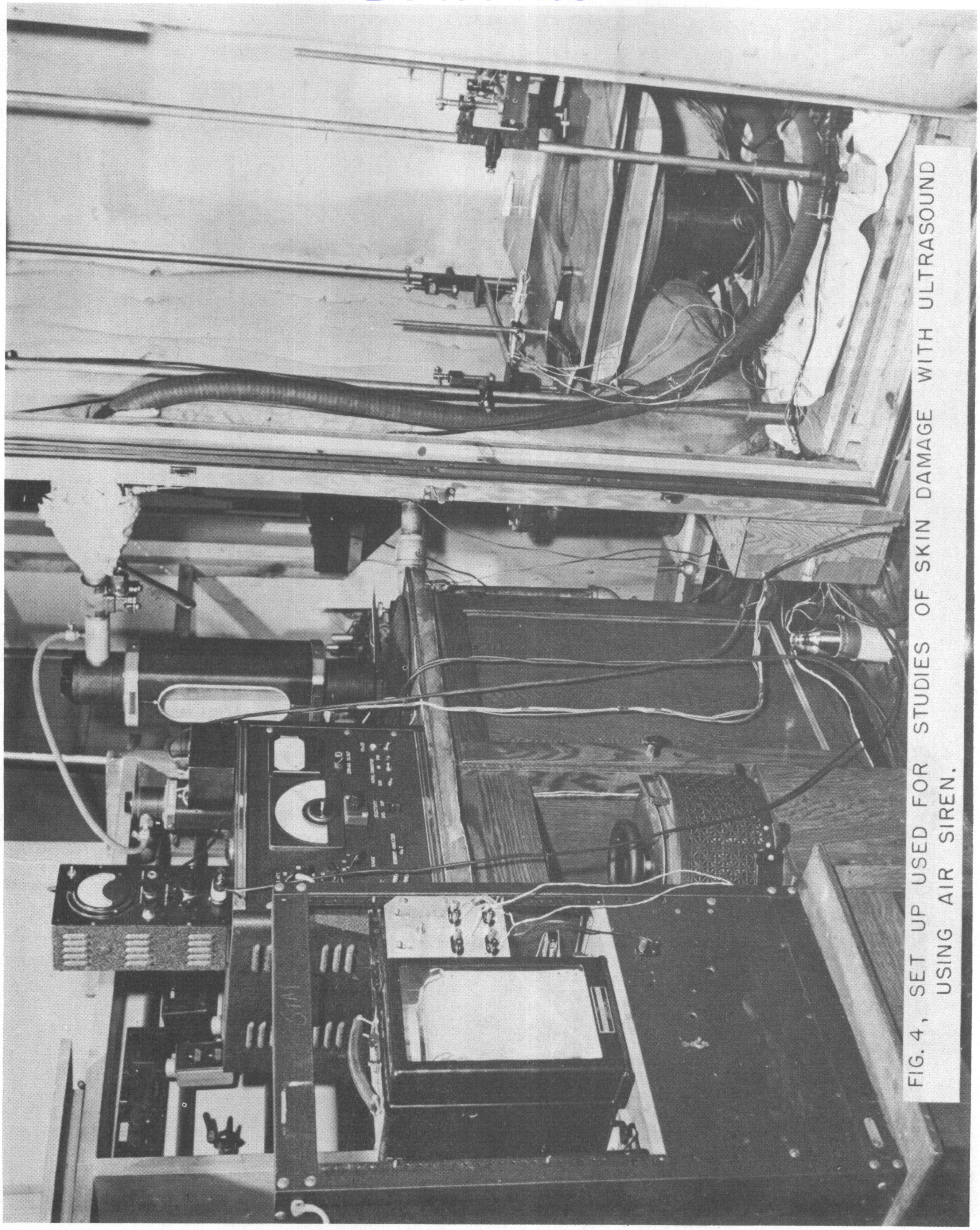


FIG. 4, SET UP USED FOR STUDIES OF SKIN DAMAGE WITH ULTRASOUND USING AIR SIREN.

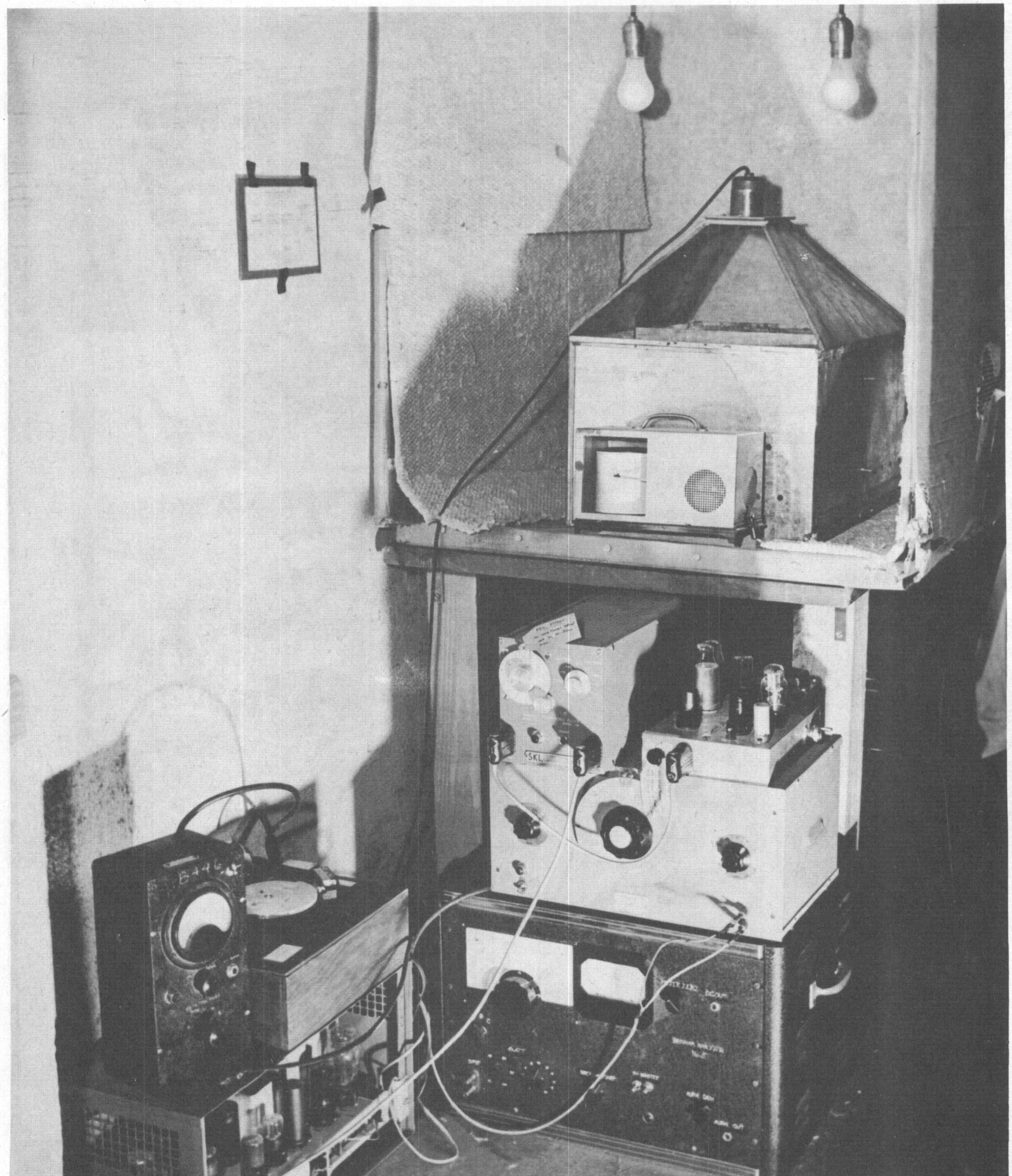


FIG. 5, APPARATUS USED FOR EXPERIMENTS WITH MODERATELY INTENSE NOISE (110db, 10-20Kc).

noise in animals. This was designed to produce intense audible noise having pressure levels of approximately 140 db (re 0.0002 dyne/cm^2) in the frequency range of 300-4800 cps. The speaker system consists of a white noise generator (or tape recording of a turbo-jet engine), a power amplifier, and a 700 watt University, B-24, loudspeaker. The latter is mounted on a large reverberant chamber which is used to house the animals during noise exposure. Details of its construction plans and octave band analyses of noise fields employed are included in the APPENDIX. Fig. 6 shows the University B-24 speaker and reverberant chamber in an air conditioned, sound insulated, room built in the animal laboratory. Fig. 7 is an outside view of the sound insulated room showing the Ampex tape recorder and automatic timer in the foreground.

d) Corona-type loudspeaker system (ionophone): Speaker systems were also constructed for use in animal experiments involving the use of intense noise at high frequencies. This work involved: the construction of 60 watt and 1.3 kilowatt corona type speakers; an investigation of the principle mechanism of sound generation; determination of the factors limiting the efficiency of these speakers, and measures of the optimum response attainable with various speaker models. Analyses of noise fields used in noise studies with the ionophone are included in the APPENDIX. Fig. 8 shows the corona-type loudspeaker set-up employed for stress studies of high intensity, high frequency noise. Fig. 9 is a close-up of the speaker unit mounted above a reverberant animal exposure chamber.

Laboratory facilities:

The following is a brief description of the laboratories in which the research was done and special equipment used for histochemical and biochemical analyses of the biological effects of noise in animals.

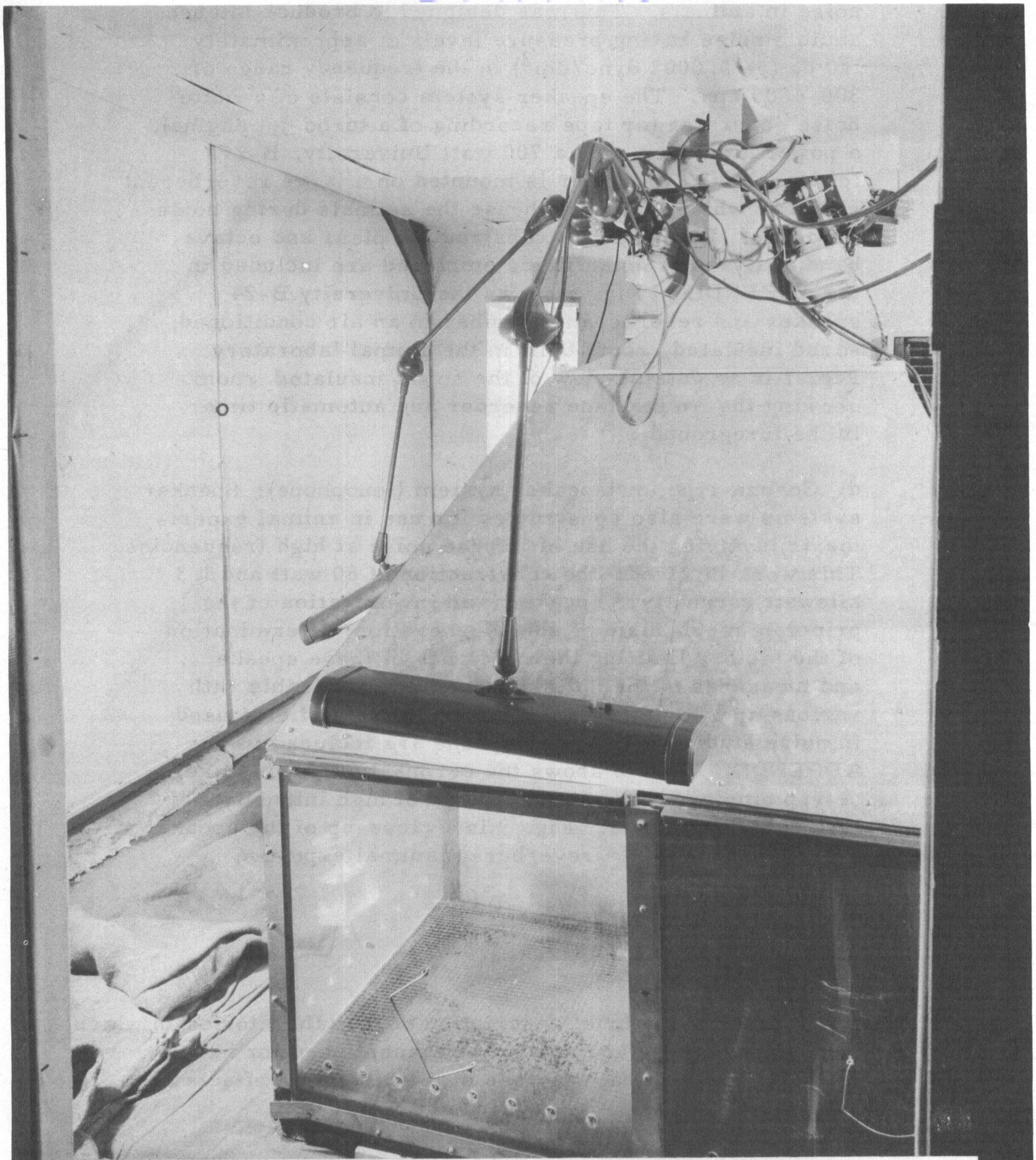


FIG. 6, INSIDE VIEW OF SOUND INSULATED ROOM SHOWING 700 WATT UNIVERSITY LOUDSPEAKER AND REVERBERANT EXPOSURE CHAMBER.

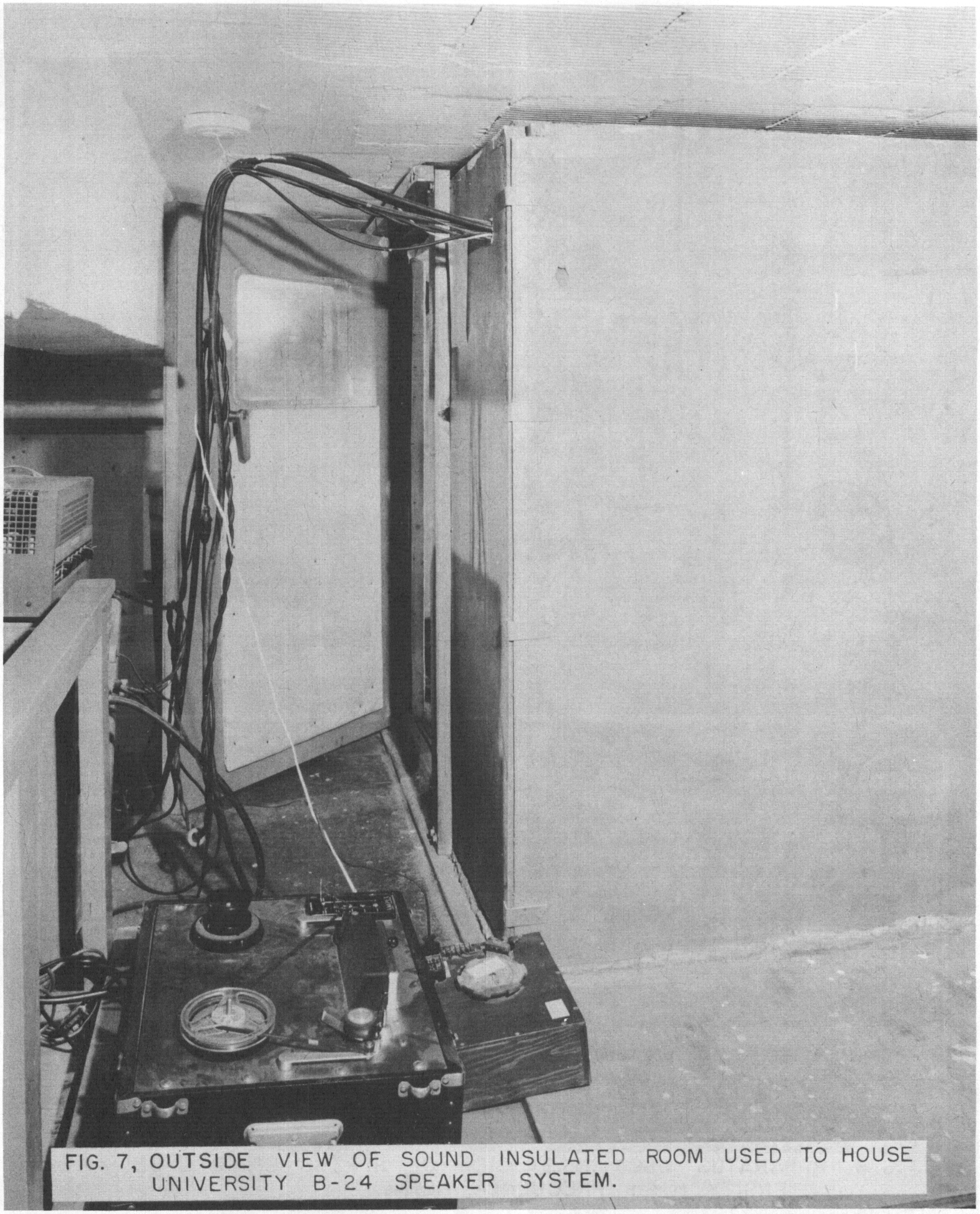


FIG. 7, OUTSIDE VIEW OF SOUND INSULATED ROOM USED TO HOUSE UNIVERSITY B-24 SPEAKER SYSTEM.

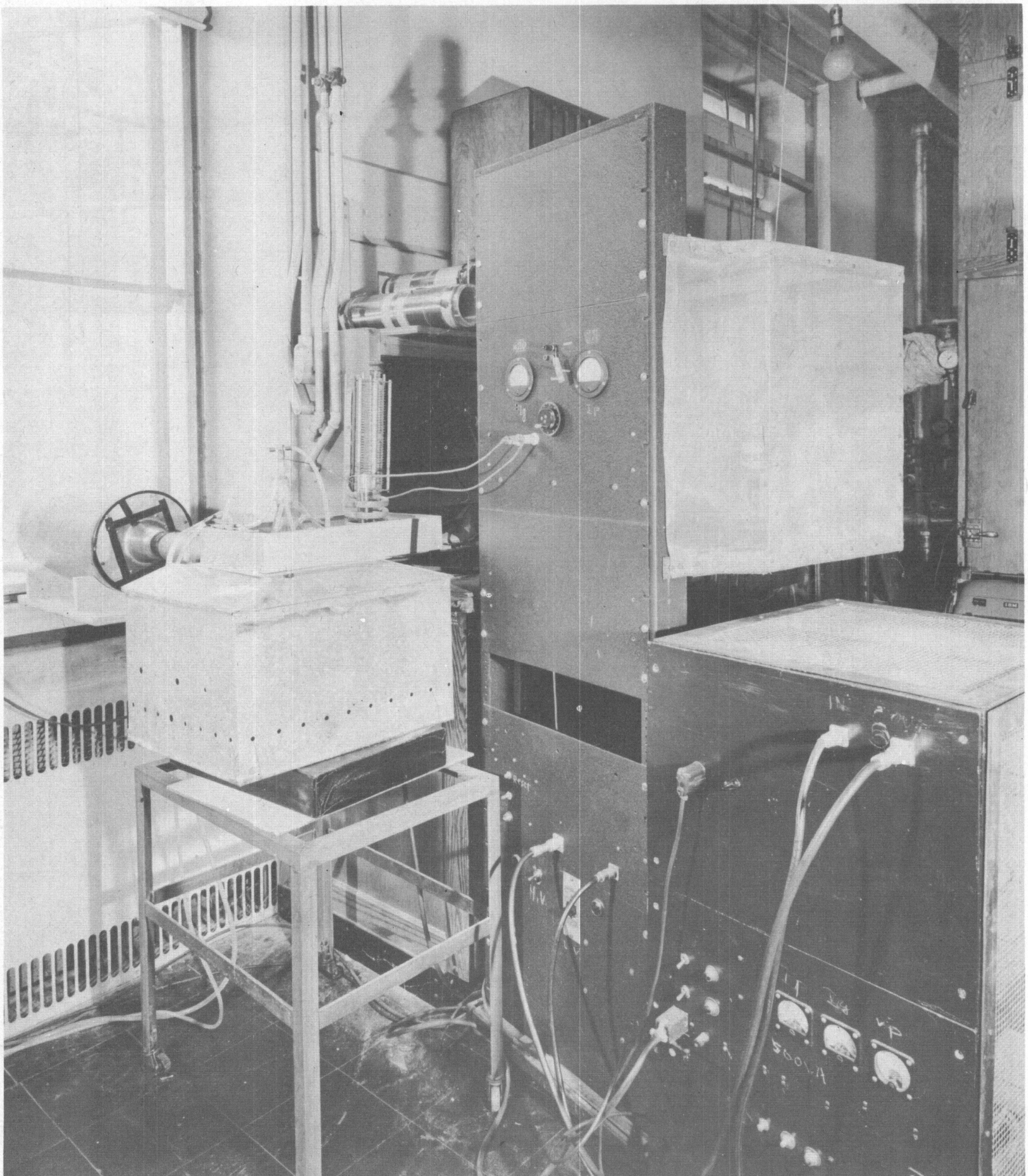


FIG. 8, APPARATUS USED IN STUDIES OF STRESS EFFECTS OF HIGH INTENSITY, HIGH FREQUENCY NOISE.

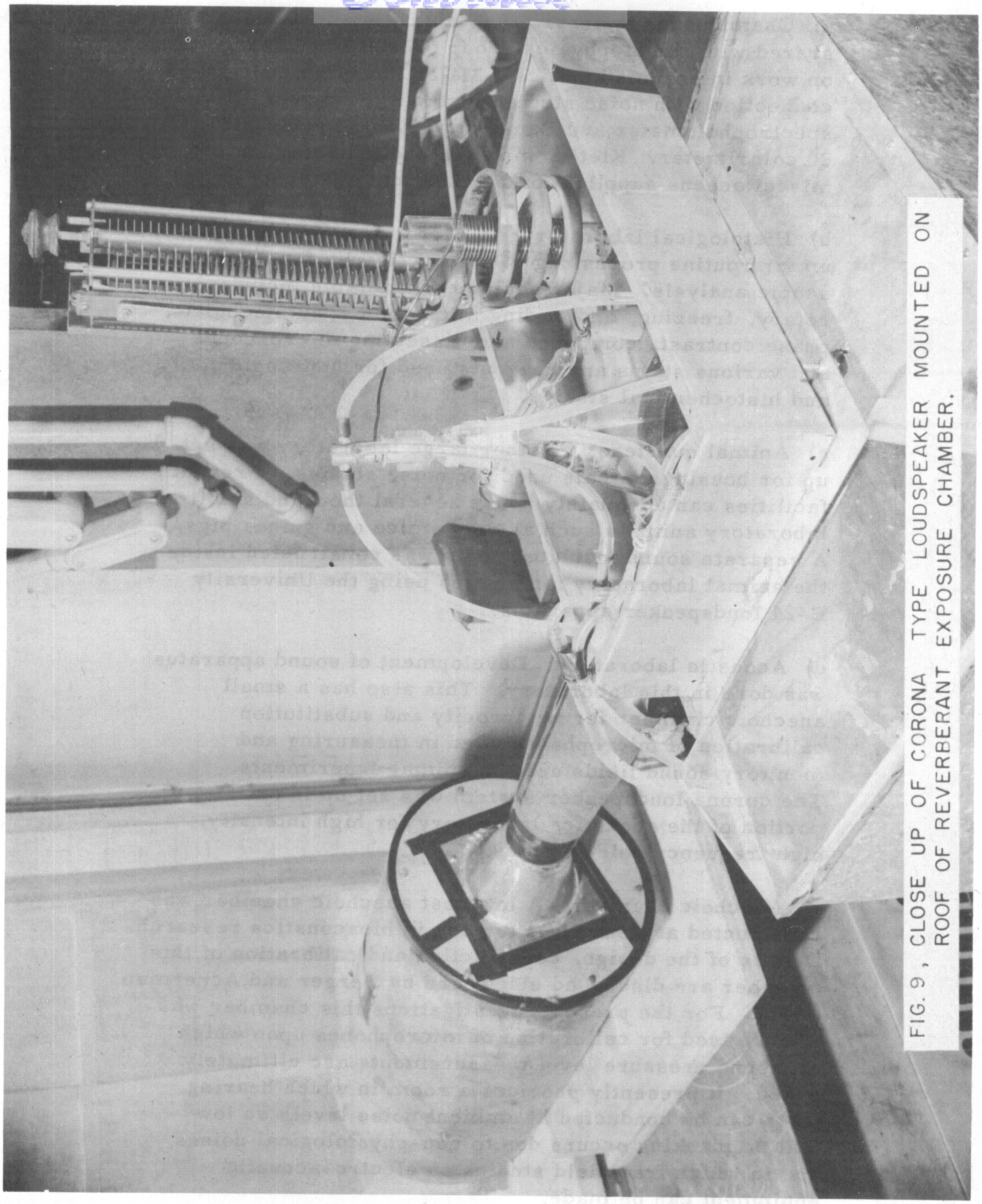


FIG. 9, CLOSE UP OF CORONA TYPE LOUDSPEAKER MOUNTED ON ROOF OF REVERBERANT EXPOSURE CHAMBER.

a) Chemical laboratory: This laboratory is actually shared with the biophysics group presently carrying on work in enzyme kinetics. Major facilities used in connection with noise studies include: Beckman DU spectrophotometer and Bausch and Lomb Spectronic-20 colorimeter. Klett Sommerson colorimeter and miscellaneous supplies for biochemical analyses.

b) Histological laboratory: A separate room was set up for routine processing of animal tissues for microscopic analysis. Major equipment available includes: rotary, freezing, and sliding microtomes; autotechnicon, phase contrast, compound and dissecting microscopes and various stains and reagents used for histologic and histochemical studies.

c) Animal quarters: Another large laboratory was set up for housing animals used for noise studies. Present facilities can adequately house several thousand small laboratory animals such as rats, mice and guinea pigs. A separate sound insulated room was constructed inside the animal laboratory for studies using the University B-24 loudspeaker apparatus.

d) Acoustic laboratory: Development of sound apparatus was done in this laboratory. This also has a small anechoic chamber for reciprocity and substitution calibration of microphones used in measuring and monitory sound fields used in animal experiments. The corona loudspeaker system was set up in one portion of the acoustics laboratory for high intensity, high frequency noise studies.

e) Anechoic chamber: A low cost anechoic chamber was constructed at Penn State for use in bioacoustics research. Details of the design, construction and calibration of this chamber are discussed elsewhere by Berger and Ackerman (1956). For the present investigations this chamber was mainly used for calibration of microphones upon which all sound pressure level measurements are ultimately based. It presently provides a room in which hearing tests can be conducted at ambient noise levels so low that no masking occurs due to non-physiological noises, and in which free-field studies on electro-acoustic equipment can be made.

General Approaches:

Various noise stimuli were used to investigate the physiological effects of noise in animals. These fall into three main categories: (1) moderately intense noise of about 110 db in the 10-20 kc frequency band; (2) intense noise at lower frequencies (140 db, 300-4800 cps) and (3) intense noise at higher frequencies (132 db, 1-40 kc). Responses of animals to single noise bursts as well as the effects of chronic noise exposure were studied under various conditions.

Animals used included Swiss albino mice, and genetically inbred strains of rats and guinea pigs. The details of the experimental set-ups and numbers of animals used are listed separately for each subproject in the RESULTS section.

Specific work carried on during the course of the contract period included the following:

(1) Studies of gross and histologic changes in the adrenals.
(2) Histochemical studies of adrenal glands.
(3) Spectrophotometric analyses of serum electrolytes.
(4) Use of the eosinopenic index as a measure of adrenal activity.
(5) Quantitative analyses of behavior changes in animals.
(6) Biochemical analyses of serum ascorbic acid and blood sugar.
(7) Biochemical analyses of serum and adrenal cholesterol.
(8) Histologic studies of endocrines and other target organs influenced by adrenal secretions.
(9) Comparative effects of intense noise at high and low frequencies. on audiogenic-seizure-susceptible and seizure-resistent strains of mice.
(10) Stress effects of intense noise in mice kept on a restricted feeding regimen.

IV. RESULTS

IV. 1 STUDIES USING VERY INTENSE AIR-BORNE SOUND

Purpose:

These studies were undertaken as a further contribution toward the understanding of primary (direct) and secondary (systemic) effects of skin exposure to intense air-borne sound. Most of this work was done under contract AF 33(038)-786. Detailed analysis of adrenal changes were completed under the present contract AF 33(616)-2505.

A brief summary of the adrenal response which occurs as a consequence of sonic induced skin burns is included at this point. This will provide a basis for comparing noise induced adrenal changes with those following sonic burns. There is no question that the latter exemplify a severe stress situation.

Procedure:

The sound source was the powerful air siren described by Allen and Rudnick (1947). Frequencies used were in the ultrasonic range for humans (18-20 kc) with sound pressure levels from 150-168 db (re $2 \times 10^{-4} \mu$ bar). Mice were completely shielded from the sound except for a small ventral abdominal area of the skin (0.5 in. diameter). Details of the experimental procedure are described by Anthony and Danner (1955) and Anthony (1956).

Summary of Results:

Systemic effects in hairless mice were studied following abdominal skin exposure to intense air-borne sound. Comparisons were made between effects of intense sound, i.e. 150 db, 18 kc and 160-168 db, 20 kc. It was found that a measurable increase in adrenal response occurred in mice exposed to sound pressure levels of about 150 db. No evidence was obtained to indicate that skin irritation with sound at this level was harmful to the mice.

In other studies it was found that skin irradiation with higher sound pressure levels (160-168 db) resulted in local burns. The systemic response was typical of changes expected following severe or harmful stress stimuli. These results are summarized in detail by Anthony (1956).

It is pertinent at this point to briefly compare the difference in adrenal response to a stress stimulus to which an animal can adapt from the adrenal changes which occur in response to a damaging stress stimulus. Fig. 10 is an adrenal taken from a mouse having a severe sonic induced skin burn. Fig. 11 shows the microscopic picture of an adrenal of a mouse following skin irradiation with ultrasound where no skin heating occurred. Both adrenals were stained with Sudan Black which is specific for lipids. Since the adrenal corticoids are lipoprotein in nature, this histochemical stain permits the localization of adrenal hormones in the cortex. The adreno-medullary junction is less distinct in Fig. 10 so the approximate extent has been outlined with a black line. It is evident that there is a marked vacuolization of the adrenal cortex in response to sonic induced skin burns. This is the adrenal picture one finds in animals in a state of shock following exposure to a severe stress situation. Fig. 11, on the other hand, presents the adrenal response which typifies an adaptive reaction to a mild stress. The adrenal cortex is well delineated from the medulla and is intensely sudanophilic in response to increased adrenocorticotropic stimulation by the anterior pituitary.

It is noteworthy that in all of our noise experiments discussed in the following sections the adrenal response was similar to that shown in Fig. 11, i.e. an adaptive response rather than one that indicated the tolerance of the animal was being exceeded. The only instance where excessive depletion of adrenocortical elements occurred was when noise stimulation was coupled with the added stress of food restriction.

IV. 2. EFFECTS OF NOISE ON EOSINOPHILS OF MICE

In the following experiments animals were exposed to various types of noise stimuli and studies made of blood changes, biochemical changes in the adrenals and changes in target organs influenced by adrenocortical secretions. These studies were designed to determine the extent of pituitary-adrenal activation which follows noise stimulation. This would in turn provide a measure of the effectiveness of noise as a stress stimulus.

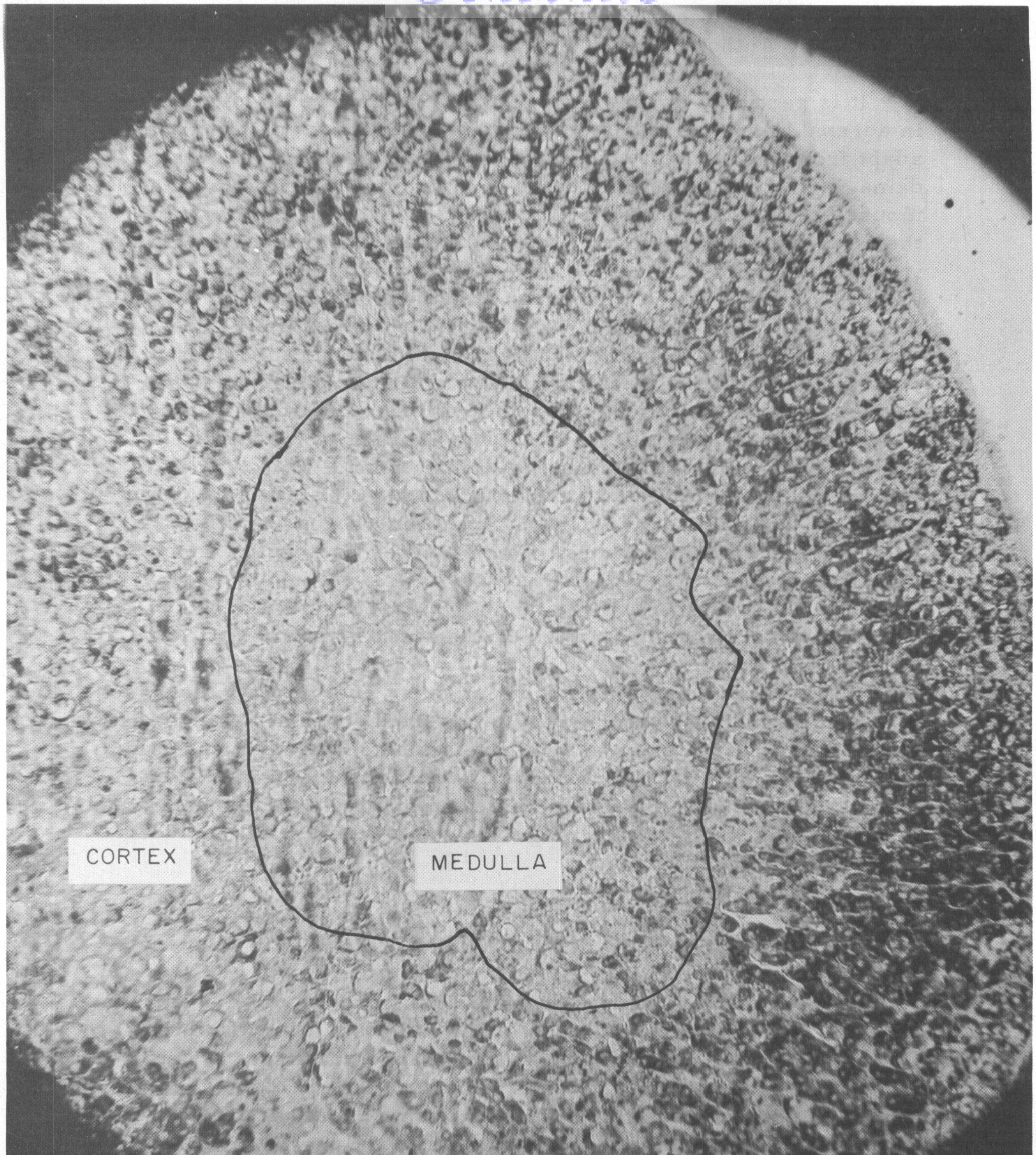


FIG. 10 - ADRENAL FROM MOUSE WITH SONIC SKIN BURN SHOWING MARKED VACUOLIZATION OF CORTEX. SUDAN BLACK STAIN.

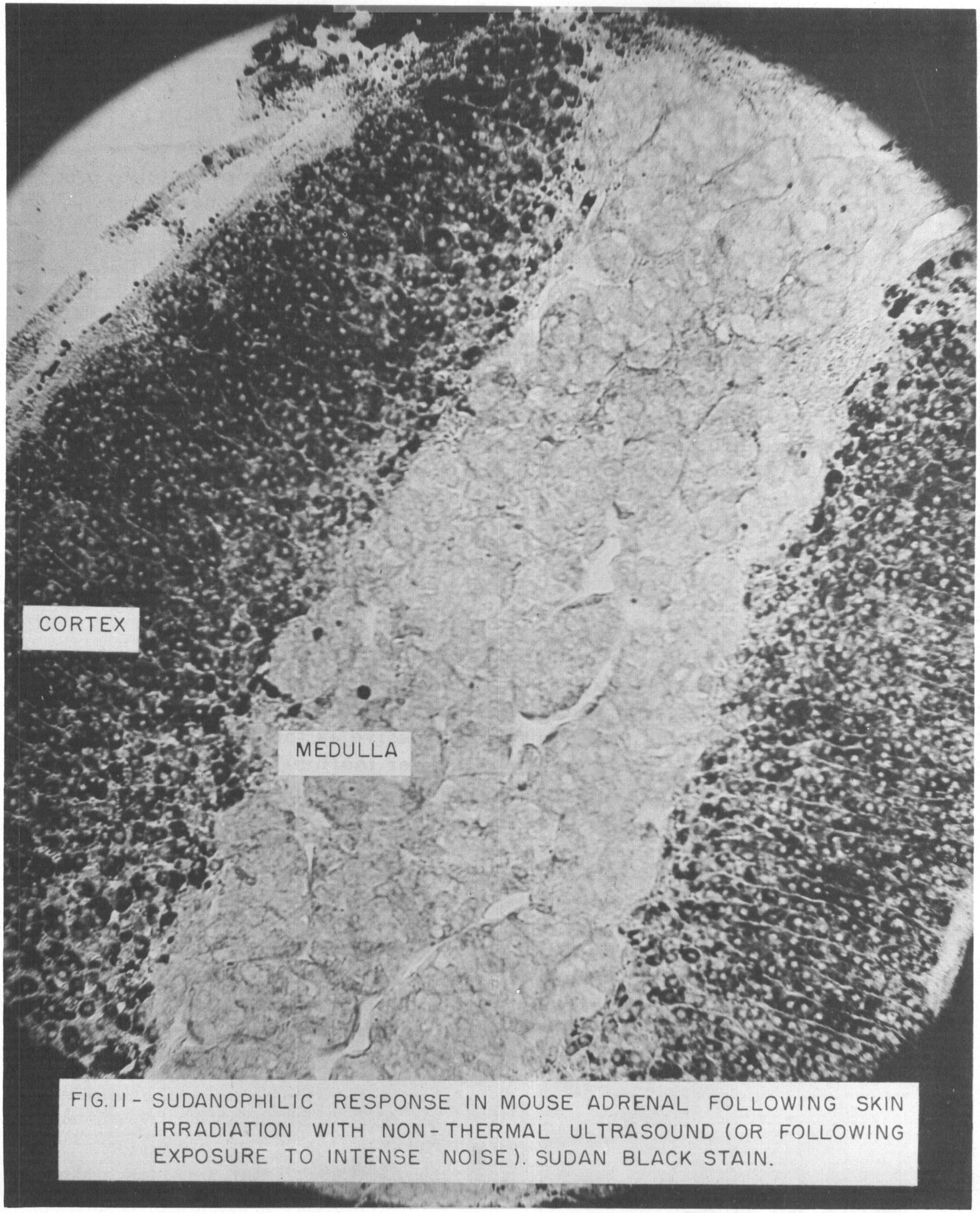


FIG. 11 - SUDANOPHILIC RESPONSE IN MOUSE ADRENAL FOLLOWING SKIN IRRADIATION WITH NON-THERMAL ULTRASOUND (OR FOLLOWING EXPOSURE TO INTENSE NOISE). SUDAN BLACK STAIN.

A. Effect of Moderately Intense Noise (110 db, 10-20 kc) on Blood Eosinophils of Mice

Purpose:

These studies were designed to determine the stress effects of prolonged exposure of mice to intermittent noise of moderately intense sound pressure levels. Since the results are discussed in more detail elsewhere (Anthony, et al, 1955) only the major findings will be summarized.

Procedure:

A total of 48 male mice of two different strains (Jax A and C57 BL/6), 45-50 days of age were used in one experiment where mice were exposed in groups of 12 to intermittent noise (seven 100 minute exposures per day). In a second set of experiments, 72 male C57 BL/6 mice, 50 days of age were housed separately and exposed to single noise bursts for 15 minutes/day for a duration of one month.

Results:

When mice were housed in groups of 12, physical contacts in Jax A males had a more marked effect on adrenocortical activation and eosinopenia than intermittent noise stimulation (Fig. 12). When fighting did not present an added variable, as in C57 BL/6 mice which are not as aggressive as the Jax A strain, intermittent noise resulted in lower morning eosinophil levels in noise exposed animals as compared to controls (Fig. 13).

Single daily exposures of C57 male to noise caused a temporary eosinopenia three hours after noise stimulation (Fig. 14).

No evidence was obtained of permanent adrenal hypertrophy, of thymicolymphatic involution or other tissue injury. Since the observed changes were transient, of short duration and no evidence of systemic pathology could be detected, it was concluded that the noise stimulus could not be considered harmful.

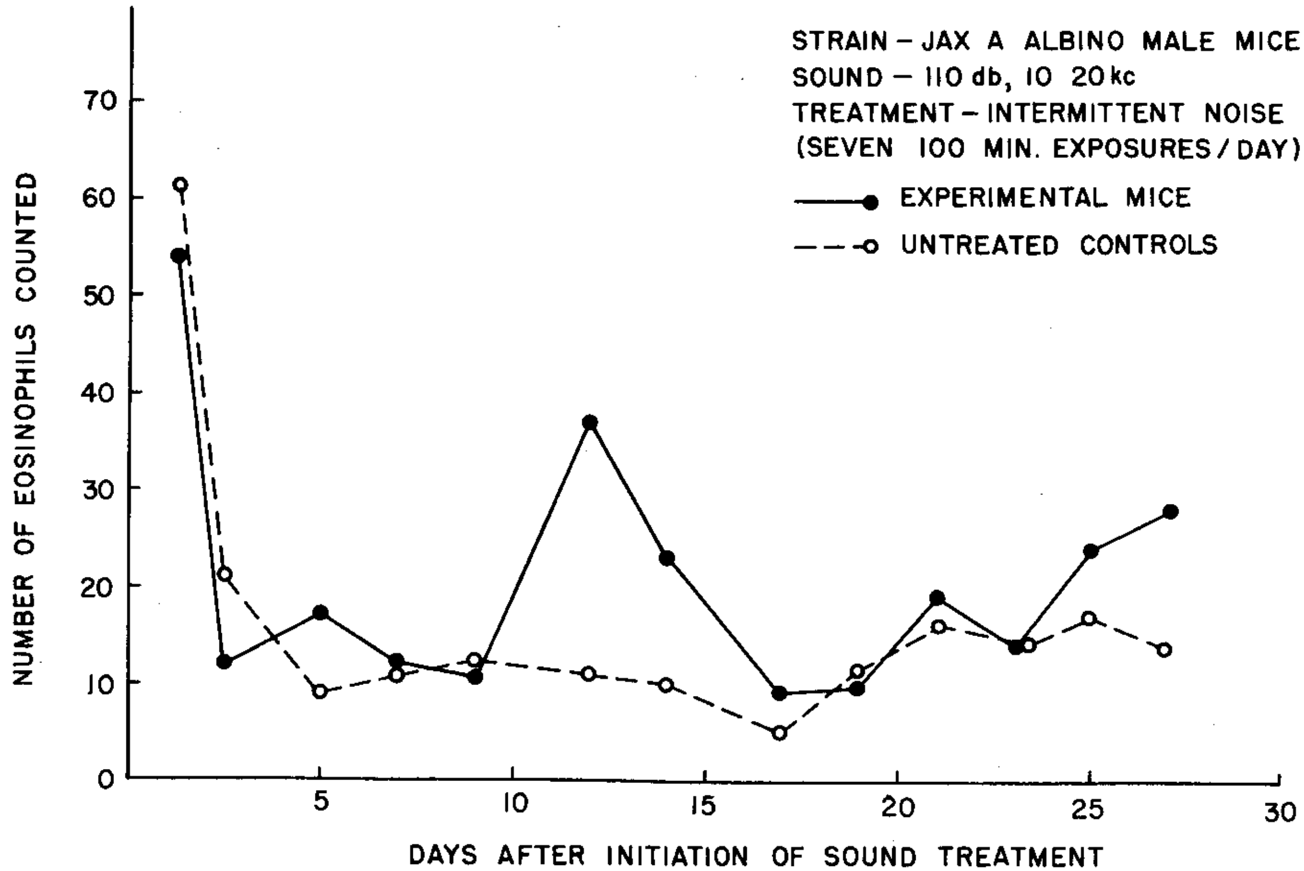


FIG. 12, MORNING EOSINOPHIL LEVELS OF CONTROL MICE AND MICE EXPOSED TO INTERMITTENT NOISE (JAX A ALBINO STRAIN).

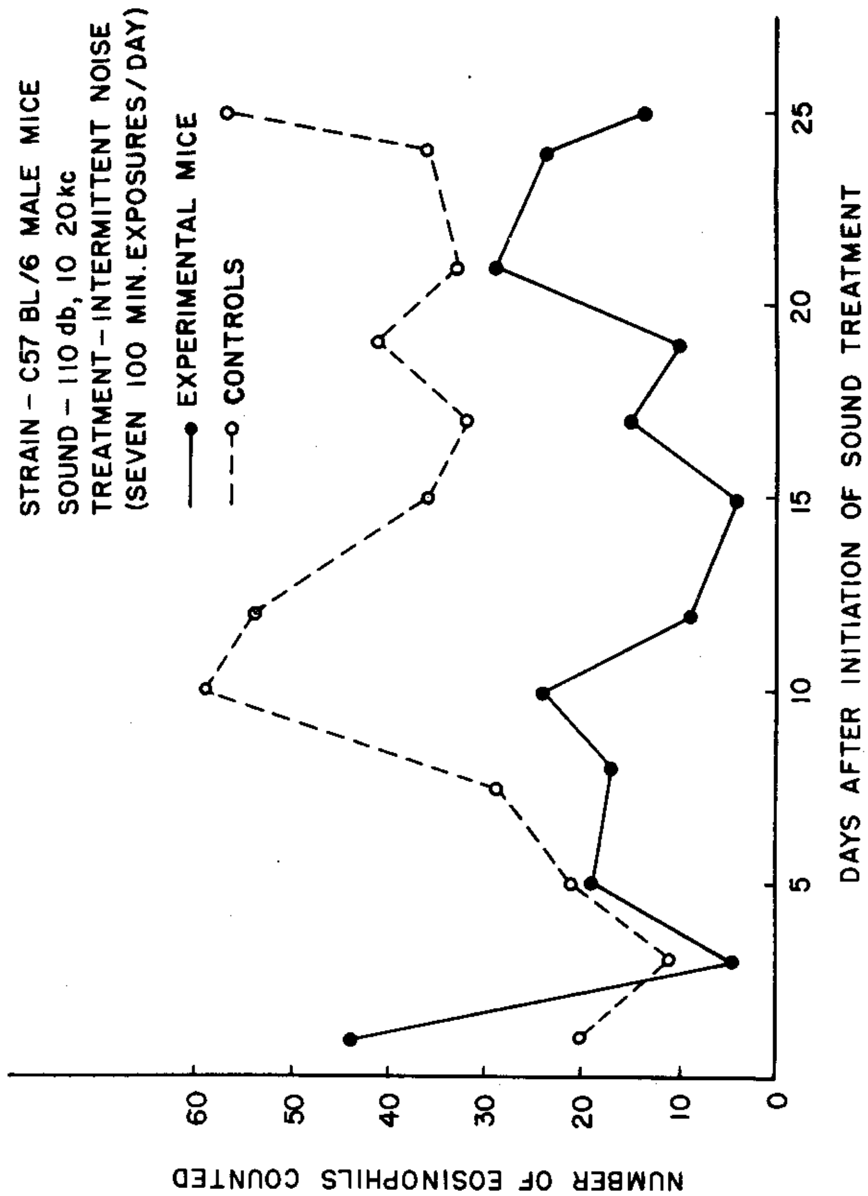


FIG. 13, MORNING EOSINOPHIL LEVELS OF CONTROL MICE AND MICE EXPOSED TO INTERMITTENT NOISE (C57BL/6 STRAIN).

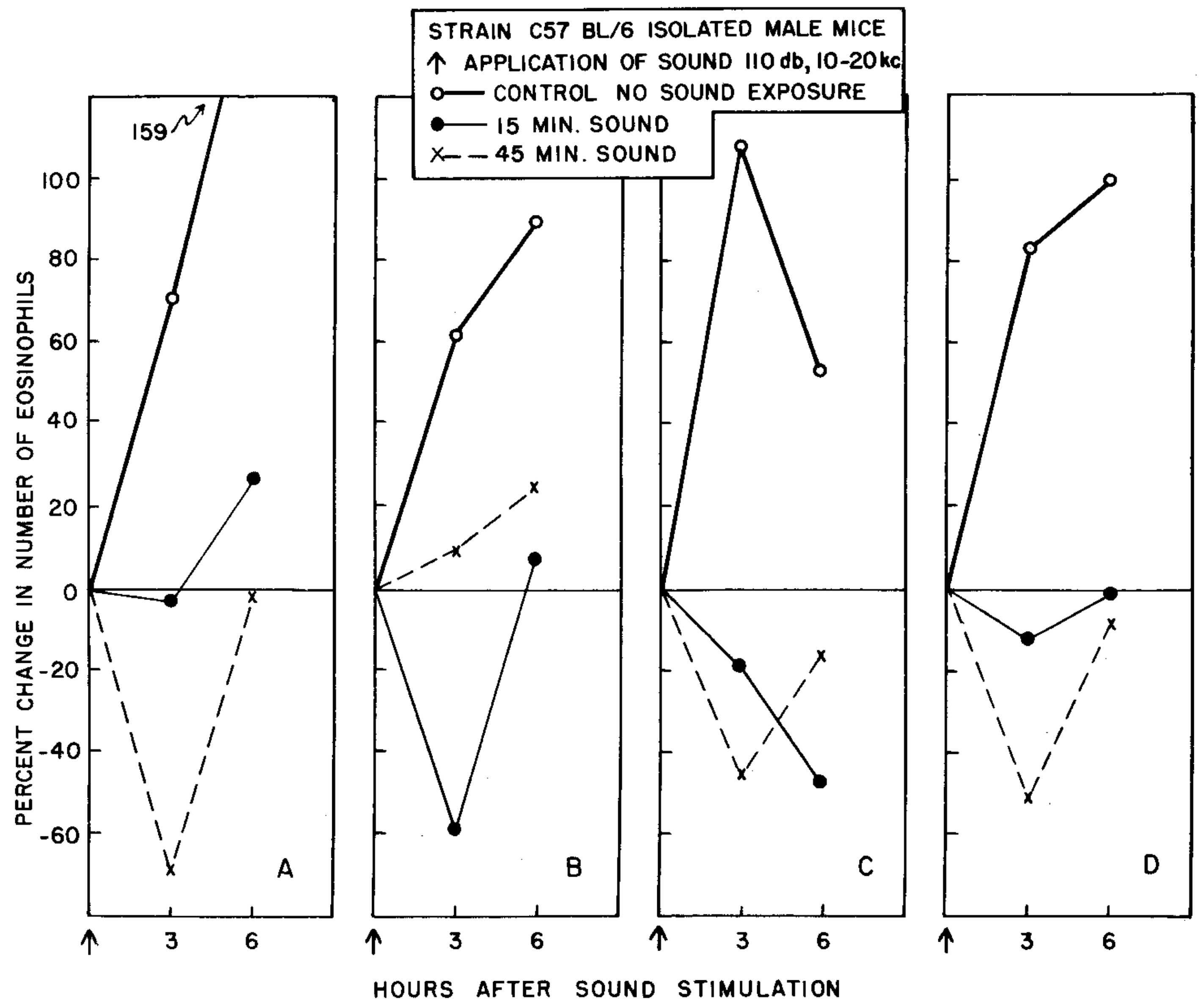


FIG. 14, EOSINOPHIL CHANGES IN UNTREATED AND SOUND EXPOSED MICE. GRAPHS A, B, C AND D MEASURE RESPONSES ON THE 11th, 17th, 25th AND 56th DAY OF EXPERIMENT. CONTROLS EXHIBIT DIURNAL CHANGES ON THESE DAYS.

B. Effects of Moderately Intense Noise on Eosinophils of Audiogenic-Seizure-Susceptible and Seizure Resistant Mice

Purpose:

These studies were done to examine the stress effects of auditory stimulation on eosinophils of mice selected on the basis of their susceptibility to audiogenic seizures. Since these results are also discussed elsewhere (Anthony, 1955), only the major findings will be summarized.

Results:

Sound pressure levels used were about 110 db re 0.0002 μ bar in a 10-20 kc frequency band. Daily exposures of seizure-susceptible and seizure-resistant mice does not abolish or alter the normal diurnal rhythm in circulating eosinophils, provided a recovery period is allowed between the last noise stimulation and the time of noise testing. A single noise stimulus is followed by a moderate eosinopenia in seizure-resistant mice (Fig. 15) and a more marked eosinopenia in seizure-susceptible mice (Fig. 16) in about 3 hours; recovery occurs in both cases within 24 hours. A prolonged eosinopenia occurs following several successive noise bursts - at a moderately low level (ca 250 eosinophils/cu mm blood) in mice which experience convulsions during sonic treatment (Fig. 17).

It was concluded that noise stimulation acts as a mild stress in both seizure and non-seizure mouse strains and is harmful only when it results in the production of fatal convulsions.

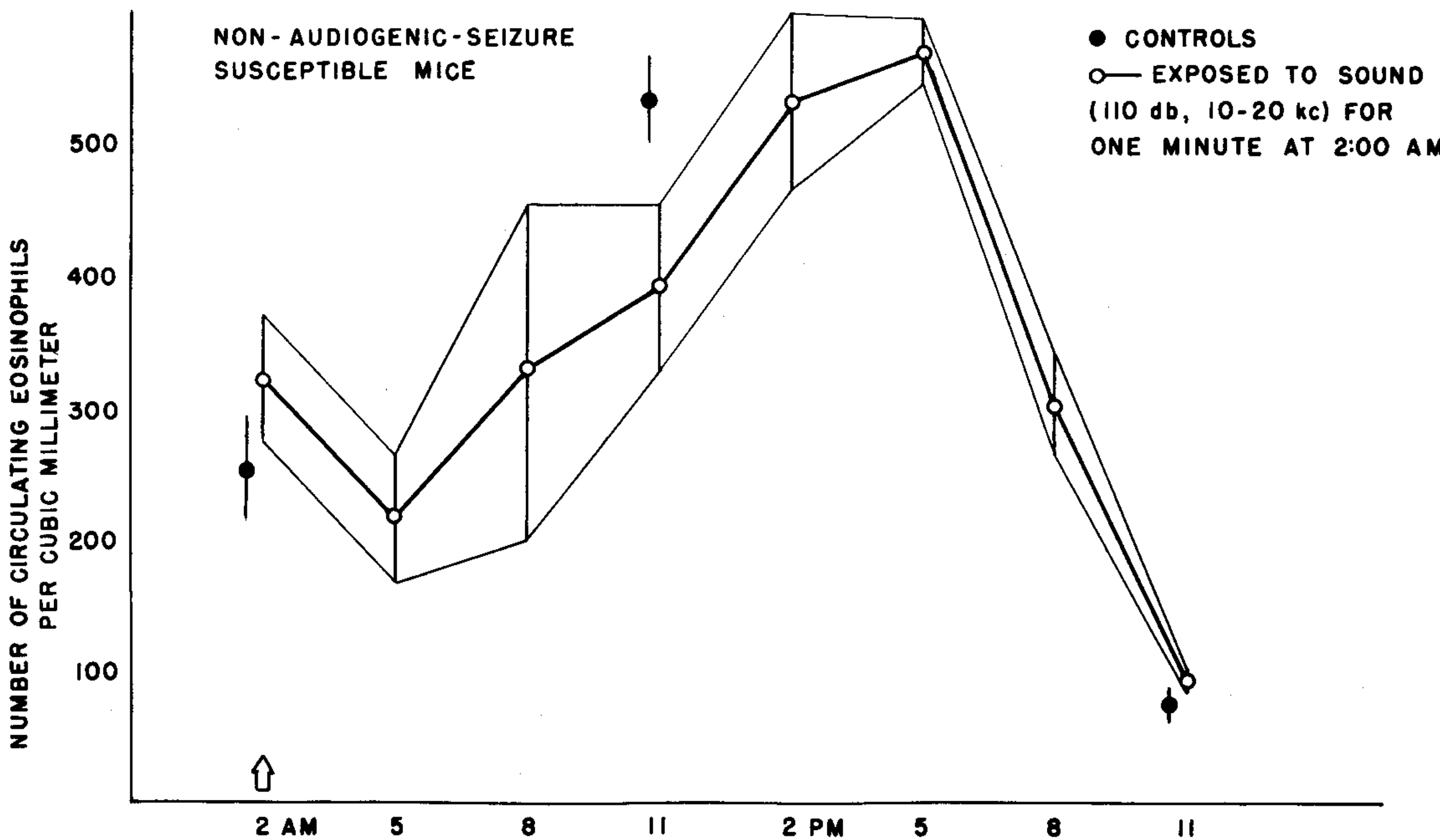


FIG. 15, EFFECT OF SINGLE NOISE STIMULUS ON EOSINOPHILS OF SEIZURE-RESISTANT MICE.

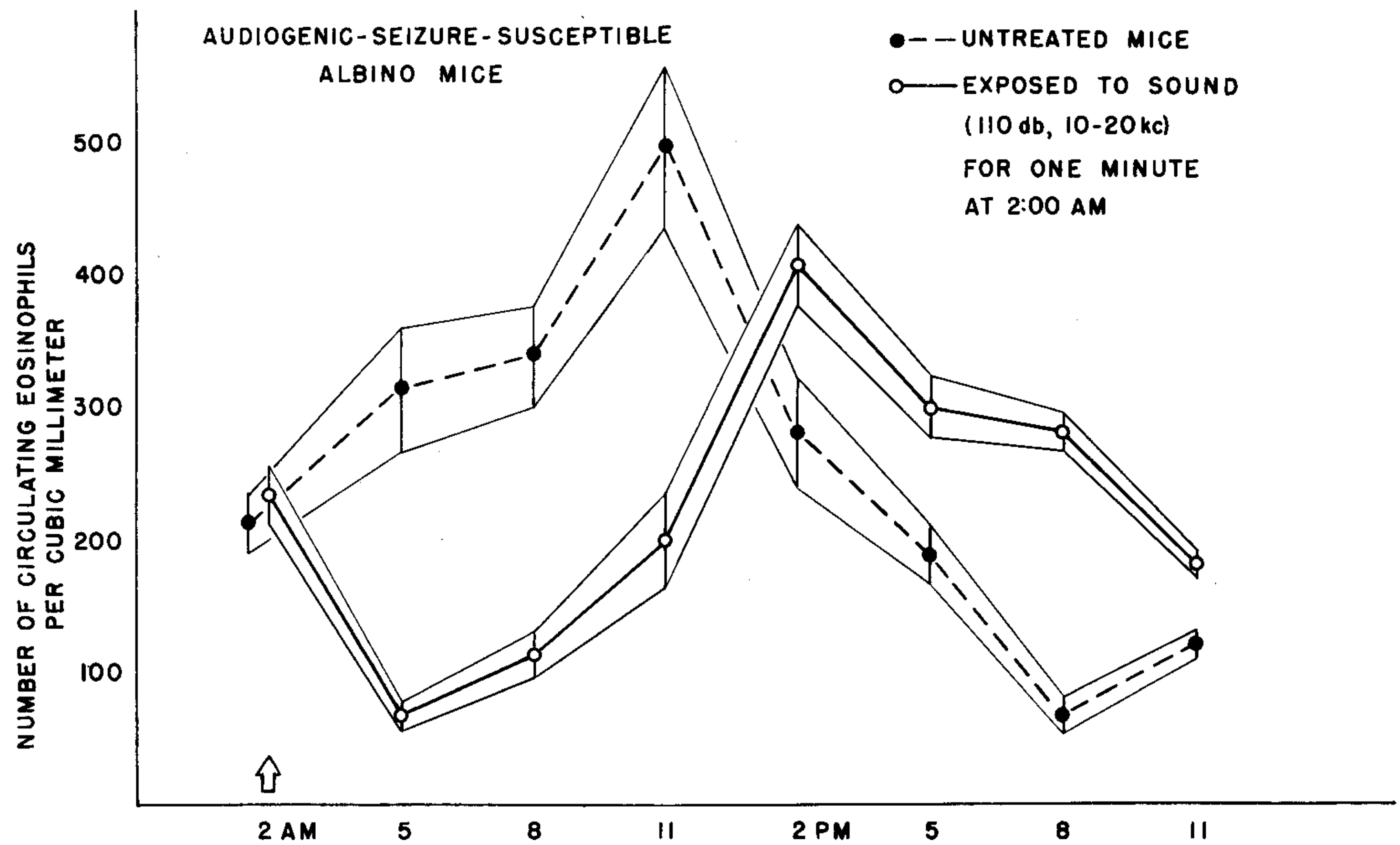


FIG. 16, EFFECT OF SINGLE NOISE STIMULUS ON EOSINOPHILS OF AUDIOGENIC-SEIZURE-SUSCEPTIBLE MICE.

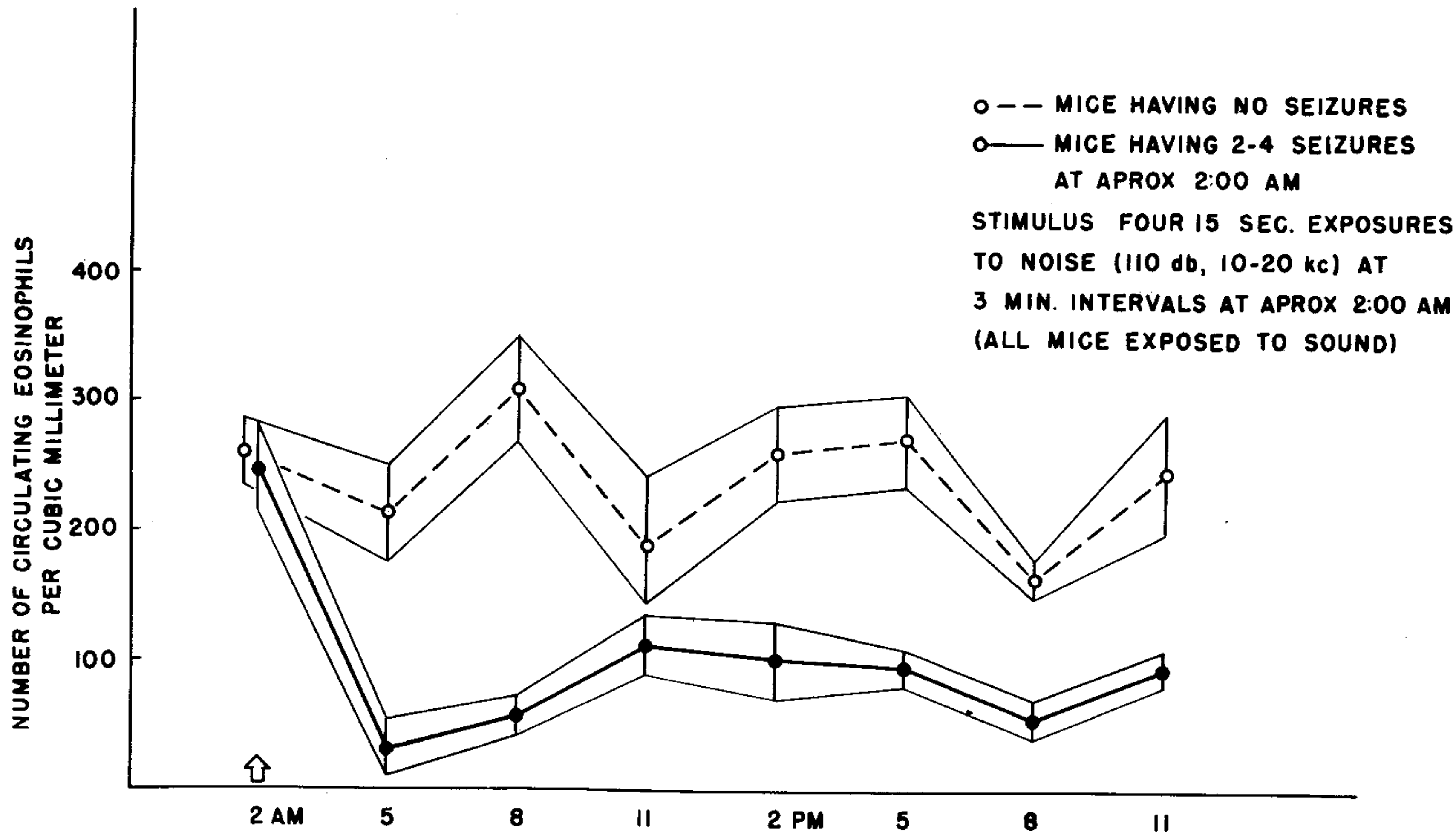


FIG. 17, EFFECT OF SEVERAL SUCCESSIVE NOISE STIMULI ON EOSINOPHILS OF SEIZURE-SUSCEPTIBLE AND SEIZURE RESISTANT MICE.

C. Eosinophil Response of Mice to Single Intense Noise Burst (139 db) in the 300-4800 cps Frequency Range

Purpose:

In the next series of experiments it was decided to examine the stress effects of more intense noise (132-139 db) using two frequency ranges - a low frequency (300-4800 cps) and a high frequency (2-30 kc). The eosinophil response was again used as an index of adrenocortical activation so that the results could be compared with data from earlier studies where moderately intense noise levels (ca 110 db) were employed.

Procedure:

In this study 86 male mice were equally divided into two groups. One group was exposed to 6 minutes of a tape-recorded jet engine noise (139 db, 300-4800 cps) at 9:00 A. M. using the B-24 loudspeaker system. The control group was subjected to an equivalent amount of handling but received no noise stimulation. Both groups were housed in similar cages which were partitioned in order to keep all mice indivually isolated. Tail blood samples were taken from six noise exposed and six control mice at zero time (9 A. M.) and eosinophil counts made. This was repeated over four hour intervals over a twenty-eight hour period.

Results:

The results of these studies are included in Table 1 and are graphically summarized in Fig. 18. It can be seen from Fig. 18 that intense noise exposure was not followed by an eosinopenia. On the contrary, the mean eosinophil level of noise exposed mice four hours after stimulation was appreciably higher than that found in controls. The pattern of eosinophil changes 8-15 hours after stimulation shows the typical compensatory increase in eosinophil levels with a return to lower levels after 24 hours. The fluctuation in eosinophil levels of controls confirms previous findings that there is a diurnal rhythm normally. The reason peaks and low points obtained in the control mice of the present study do not correspond to those reported in earlier work (Fig. 16) is that a shorter period of equilibration of mice was used prior

Table 1. Mean Eosinophil Counts from Mice Exposed to Intense Noise (139 db, 300-4800 cps).

	Mean No. Eosinophils ± Exposed	Control
9:00 A.M.	165 ± 15	135 ± 11
1:00 P.M.	190 ± 17	75 ± 13
5:00 P.M.	330 ± 46	130 ± 18
9:00 P.M.	215 ± 9	175 ± 18
1:00 A.M.	370 ± 72	220 ± 86
4:00 A.M.	165 ± 18	305 ± 36
7:30 A.M.	55 ± 12	110 ± 26
10:30 A.M.	60 ± 13	25 ± 4

* Values recorded in cells per cu mm of blood plus-minus standard deviation of the mean. Each value represents mean obtained from 6 mice.

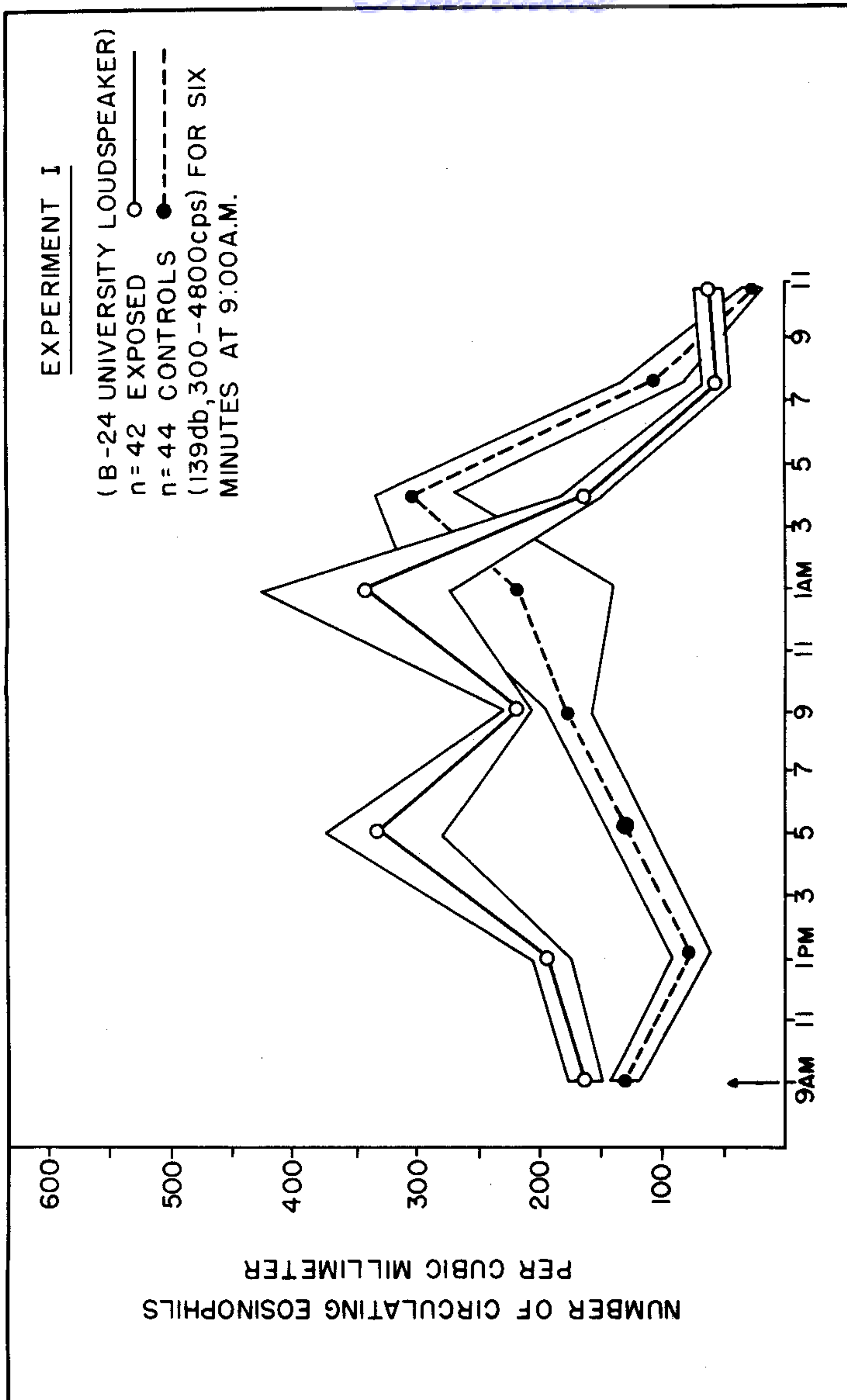


FIG.18, EFFECT OF SINGLE NOISE STIMULUS ON EOSINOPHILS OF SEIZURE RESISTANT MALE MICE.

to the experiment. It is important to isolate animals for a period of one to two weeks if one wishes to correlate diurnal eosinophil patterns with diurnal activity. In the present experiment this was not necessary since the item of interest was the eosinopenic response to noise irrespective of what the eosinophil count was at the time of stimulation.

One is forced to conclude from this experiment that noise pressure levels of 139 db at low frequencies are no more effective than lower noise intensities (110 db, 10-20 kc) in causing an eosinophil drop in mice. The similarity in the compensatory increase in eosinophils 8-15 hours after noise exposure with intense low frequency noise with that found in earlier experiments using moderately intense noise levels suggests that the animals' adaptive response mechanisms work equally well at these two intensity levels (compare Fig. 18 with Fig. 15).

D. Eosinophil Response of Mice to Single Intense Noise Burst (132 db) at Higher Frequencies (2-40 kc).

Purpose:

This study was performed to determine if intense noise may be more effective in inducing eosinopenia at higher frequencies.

Procedure:

The noise stimulus used in this study was produced by the 1.3 kilowatt model corona loudspeaker system. The intensity level of the noise was approximately 132 db in the 2-40 kc frequency band. The length of noise exposure was six minutes. As in the previous experiment with the B-24 loudspeaker system, tail blood samples were withdrawn immediately after noise exposure for eosinophil counting. It was mainly of interest to determine the eosinopenic response three to five hours after noise stimulation. For this reason, eosinophil determinations were made on controls and non-exposed animals at zero time (immediately following noise exposure) and at three and five hour intervals thereafter.

Over three hundred male mice were used in this study. A total of two hundred eleven mice were exposed to intense noise

(132 db, 2-20 kc) for six minutes and one hundred thirty-one mice were used as controls. For practical reasons usually only 36 mice could be used for an experimental run and blood samples taken from six experimental and six controls during the three time intervals. As in previous studies no individual was used more than once for blood sampling during an experimental run.

Results:

The combined data from eight separate tests are included in Table 2 and summarized graphically in Fig. 19. It can be seen that there is again no appreciable difference in the eosinophil levels of control mice as compared to mice exposed to intense high frequency noise for six minutes. The slight drop in eosinophils which occurred in both control and experimental mice was probably due to some variable other than noise, such as handling.

Table 2. Percentage Change in Eosinophils at 3, 6 and 12 hour Intervals of Mice Exposed to Noise (139 db, 300-4800 cps)

Date	No. of Eosinophils at 0-hour	% Change at 3-hour	% Change at 6-hour	% Change at 12-hour
12/30	220 ± 27 (4) *	91 ± 7% (5)	69 ± 4% (2)	43 ± 11% (4)
12/31	230 ± 10 (3)	124 ± 8% (2)	81 ± 0% (1)	---
1/20	295 ± 52 (3)	158 ± 2% (3)	0 ± 9% (5)	66 ± 7% (4)
1/21	105 ± 7 (2)	62 ± 15% (4)	329 ± 9% (4)	243 ± 9% (4)
1/22	270 ± 25 (4)	54 ± 2% (2)	79 ± 8% (6)	26 ± 5% (4)
1/23	260 ± 22 (4)	2 ± 11% (4)	106 ± 11% (3)	79 ± 9% (3)
1/28	30 ± 9 (6)	800 ± 13% (6)	850 ± 13% (6)	233 ± 8% (6)
1/29	150 ± 32 (6)	120 ± 12% (6)	30 ± 87% (6)	47 ± 13% (6)
Average	195 ± 36	175 ± 9%	193 ± 18%	105 ± 19%

* numbers of mice used are indicated in parenthesis. Values are expressed as averages plus-minus standard deviation.

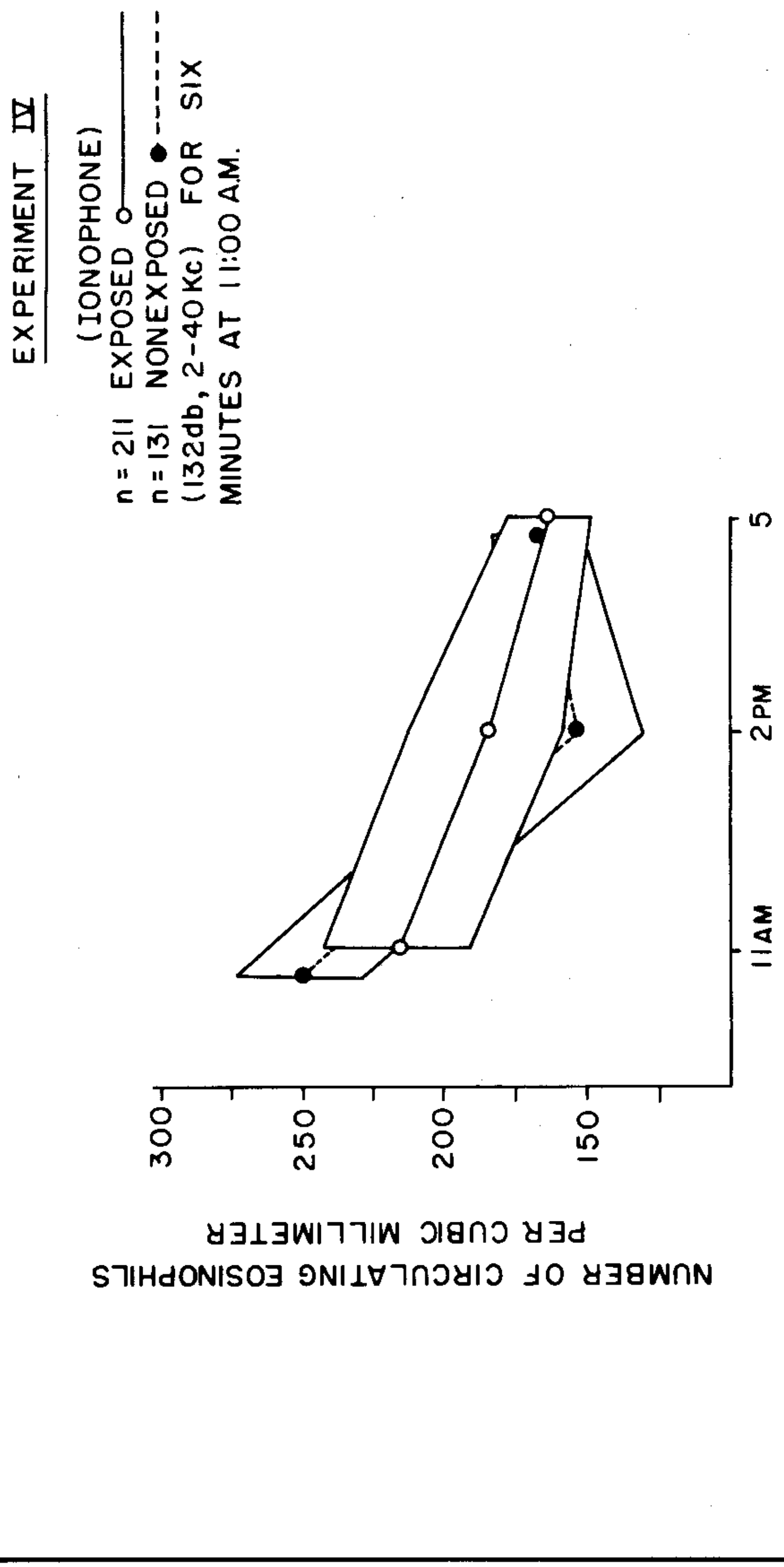


FIG. 19, EFFECT OF SINGLE NOISE STIMULUS ON EOSINOPHILS OF SEIZURE RESISTANT MALE MICE.

IV. 3. EFFECTS OF NOISE ON BLOOD CHEMISTRY

The following experiments summarize changes in various blood constituents after exposing rats, mice and guinea pigs to daily intense noise for a duration of several weeks. The sound levels used was 139 db in the 300-4800 cps frequency range. This was obtained using the University model B-24 loudspeaker system.

A. Effects of Intense Low Frequency Noise (139 db, 300-4800 cps) on Blood Serum Electrolytes

Purpose:

Studies of blood serum changes in Na and K ions following noise exposure were undertaken to determine if intense noise can cause the same pattern of ion changes reported to occur with other types of severe stress stimuli such as trauma, hemorrhage, etc.

Procedure:

Noise levels used were about 136-143 db in the 300-4800 cps frequency range. The animals used included two dozen Wistar rats, two dozen guinea pigs (Hartley strain) and two dozen Jax A albino mice. These were equally divided into control and experimental groups. The exposure time was fifteen minutes per day for a duration of approximately four weeks.

All ion analyses were made using the Beckman model DU Flame spectrophotometer with photomultiplier attachment. The analytical procedure was that described by Kingsley and Schaffert (1953).

All experimental animals were housed in a partitioned reverberant chamber in groups of six for the duration of the experiment. Serum samples were taken at the onset of the experiment and at weekly intervals thereafter.

Results:

The data from ion analyses of rats and guinea pigs are summarized in Table 3. It is evident that there is no difference between noise exposed animals and controls, either as to the mean ion values or the degree of variability. Daily exposure to intense noise under the conditions specified was apparently not effective enough as a stressing agent to elicit shifts in serum ion levels. One exception should be noted. The experimental male guinea pigs showed a significant drop in serum Na values at the termination of the experiment. However, since some of the animals also had a respiratory infection at the time of autopsy, it could not be maintained that noise was the only variable involved.

Although no differences were observed in the average ion levels of experimental and control rats, a glance at Table 3 reveals that mean ion values obtained on any one day may vary significantly from those obtained during another sampling period. This is in agreement with more extensive studies where it was shown that the normal levels of serum ions may vary considerably from day to day (Anthony and Parsons, 1957).

In the course of this particular study supplemental data were obtained on Na, K and Ca ion levels in the brain tissue of the rats, mice and guinea pigs used for serum ion analyses. The results again proved negative. No changes in brain ion levels were obtained following four weeks of daily exposure to intense noise. These data are discussed at greater length by Parsons (1956).

B. Serum Ascorbic Acid Levels of Rats and Guinea Pigs Exposed to Intense Noise (139 db, 300-4800 cps).

Purpose:

The objective of this study was to investigate possible alterations in ascorbic acid levels following intense noise stimulation. Plasma ascorbic acid changes have been reported by other investigators following severe stresses such as trauma and cold (Skelton and Fortier, 1951). It was hoped that the present study would contribute further data on the stress effects of intense noise.

Table 3. Serum Ion Levels in Rats and Guinea Pigs Following Daily Exposure to Intense Noise

Animal	Date bled		No. of daily noise exposures	Sex	Body wt. grams -	Na Meq. /l X	Na Meq. /l S. E.	Ca Meq. /l X	Ca Meq. /l S. E.	K Meq. /l X	K Meq. /l S. E.
Rat*	11/10/55	6	None	M	143 ± 17	150.7	1.7	4.77	0.19	!	!
		5	None	F	144 ± 9	153.6	1.3	4.88	0.09		
		5	None	M	173 ± 10	152.0	1.7	4.92	0.09		
		5	None	F	137 ± 9	152.0	1.8	4.76	0.04		
	Total	21				152.0	0.7	4.83	0.06		
	11/18/55	6	None	M	208 ± 33	150.7	1.7	5.13	0.10	5.82	0.09
		5	None	F	171 ± 8	153.6	1.3	4.90	0.07	5.58	0.24
		6	One	M	242 ± 9	152.0	1.7	4.75	0.09	5.62	0.11
		6	One	F	154 ± 6	152.0	1.8	4.88	0.12	5.33	0.13
	Total	23				152.0	0.9	4.92	0.05	5.59	0.08
	11/28/55	6	None	M	228 ± 36	147.0	0.7	4.97	0.13	5.82	0.15
		3	None	F	182 ± 8	146.7	1.8	4.93	0.12	6.07	0.30
		6	Eleven	M	245 ± 25	147.3	1.3	4.80	0.07	5.92	0.23
		6	Eleven	F	177 ± 10	145.3	1.2	4.92	0.03	5.43	0.26
	Total	21				146.6	0.6	4.90	0.04	5.77	0.12
	12/ 7/55	6	None	M	240 ± 36	148.7	1.0	4.97	0.05	5.85	0.16
		3	None	F	188 ± 10	148.7	1.0	5.00	0.06	5.33	0.73
		6	Twenty	M	263 ± 29	149.7	1.1	4.93	0.20	5.53	0.15
		6	Twenty	F	188 ± 14	148.7	0.7	5.22	0.12	5.37	0.18
	Total	21				149.0	0.5	5.03	0.07	5.55	0.12

* "CF-Wistar" strain

! values discarded because of hemolysis of serum sample

Table 3 (continued)

Rat	12/15/55	6	None	M	260 ± 38	155.0	1.2	4.92	0.13	4.98	0.35
		6	None	F	198 ± 12	154.7	2.0	5.12	0.11	5.33	0.51
		5	Twenty-eight	M	294 ± 20	155.6	1.0	4.90	0.12	5.26	0.25
		6	"	F	222 ± 38**	154.0	1.8	4.85	0.17	5.50	0.16
	Total	23				154.8	0.7	4.95	0.06	5.27	0.16
Grand Total		109 (88 for K)				151.0	0.4	4.93	0.03	5.54	0.07
Guinea Pig	12/17/55	6	None	M	432 ± 29	148.3	2.1	6.28	0.50	8.07	1.05
		4	None	F	406 ± 36	148.0	1.2	6.10	0.24	8.67	1.21
		5	Twenty-nine	M	368 ± 15	141.6	2.6	6.56	0.50	9.72	0.78
		5	Twenty-nine	F	410 ± 22	149.6	2.0	5.88	0.32	7.54	1.32
	Total	20				146.9	1.2	6.21	0.20	8.47	0.53

** two individuals pregnant

Procedure:

The same animals were used for this study as in the previous experiment concerned with noise and serum electrolytes. These animals were thus exposed to 15 minutes of intense noise (139 db, 300-4800 cps) for a period of about 4 weeks.

The diphenylhydrazine method of Roe and Kuether (1943) was used for ascorbic acid analyses of serum samples. Blood samples were withdrawn for analysis from the tail vein of 12 experimental and 12 control rats after 1, 11, 20 and 28 daily 15 minute exposures to intense noise. The procedure routinely followed called for alternately sampling control and experimental animals immediately after the last noise exposure. Ascorbic acid analyses on blood serum of guinea pigs were only done at the termination of the experiment (29 daily exposures to intense noise).

Results:

The serum ascorbic acid levels of rats after 1, 11, 20 and 28 daily noise exposures are summarized in Table 4. It can be seen that noise did not affect the mean levels of serum ascorbic acid of experimental rats as compared to non-exposed controls after 1-4 weeks of daily sound exposure.

The data for the guinea pigs are also included in Table 4. A significant increase in ascorbic acid was found in experimental male guinea pigs as compared to controls ($P < .05$) conversely, a slight reduction in ascorbic acid levels occurred in the serum of noise exposed females ($P < .10$).

C. Effects of Intense Noise (140 db, 150-4800 cps) on Adrenal and Plasma Cholesterol of Mice.

Purpose:

In this experiment changes in plasma and adrenal cholesterol were used as an index of adrenocortical activation through noise stimulation. Cholesterol is known to be one of the main precursors of the steroid hormones produced by the adrenal cortex.

Table 4. Serum Ascorbic Acid Levels in Rats and Guinea Pigs Exposed to High-Intensity Sound (136-143 db, 150-4800 cy/sec freq.)

Animal	N	Sex	Group	Time after onset of experiment	Number of sound exposures	Blood Serum Ascorbic Acid (mg %) Mean ± Standard Error
Rat	6		Control	1-6 hours	0	1.15 ± 0.11
Rat	6		Control	1-6 hours	0	0.57 ±0.03
Rat	6		Experimental	1-6 hours	1	1.30 ±0.10
Rat	6		Experimental	1-6 hours	1	0.64 ±0.40
Rat	6		Control	11 days	0	0.94 ±0.05
Rat	4		Control	11 days	0	0.74 ±0.08
Rat	6		Experimental	11 days	11	1.06 ±0.09
Rat	6		Experimental	11 days	11	0.65 ±0.04
Rat	6		Control	20 days	0	1.05 ±0.06
Rat	3		Control	20 days	0	0.71 ±0.14
Rat	6		Experimental	20 days	20	1.14 ±0.15
Rat	6		Experimental	20 days	20	0.56 ±0.04
Rat	6		Control	28 days	0	1.31 ± 0.12
Rat	6		Control	28 days	0	0.60 ± 0.05
Rat	5		Experimental	28 days	28	1.23 ± 0.08
Rat	6		Experimental	28 days	28	0.59 ± 0.07
Guinea Pig	6		Control	29 days	0	0.36 ± 0.09
Guinea Pig	5		Control	29 days	0	0.47 ± 0.07
Guinea Pig	5		Experimental	29 days	30	0.58 ± 0.05
Guinea Pig	5		Experimental	29 days	30	0.29 ± 0.05

An increased output of ACTH (adrenocorticotrophin) by the anterior pituitary following exposure of an animal to a severe stress stimulus is often reflected in a reduction in plasma and adrenal cholesterol within 3-6 hours after stimulation. This then can serve as another index of the stressful nature of intense noise.

Procedure:

Two noise levels were used to stimulate different animal groups. The first noise stimulus (114 db, 10-20 kc) was produced using the W.E. 713C speaker system (Fig. 5). The other noise source was produced using the University B-24 speaker system. With the latter the noise levels attained were about 140 db with most of the energy concentrated in the 150-4800 cps frequency range. Individually isolated Swiss albino mice were exposed to noise for 5 minutes and autopsied immediately after exposure and at 3 and 6 hour intervals for analysis of changes in adrenal weight and in adrenal content of cholesterol. Supplementary data were also obtained on changes in plasma cholesterol and serum Na, K and Ca levels following noise exposure.

Preliminary analyses for total cholesterol were made using the Sperry-Webb technique (1950). This procedure was later abandoned in favor of a simpler method of analysis recommended by Zlatkis, Zak and Boyle (1953).

Results:

Cholesterol levels in adrenals and plasma of control mice and mice exposed to intense noise are summarized in Table 5. It appears from Table 5 that neither the moderately intense (110 db) nor the intense (140 db) noise stimulus caused any appreciable change in adrenal or plasma cholesterol levels. The mean adrenal and plasma cholesterols of Groups I and II differ from Groups IV through VI since the first two groups were analyzed by the Sperry-Webb technique and the others by the method of Zlatkis.

Table 5. Cholesterol Levels in Adrenals and Plasma of Mice Exposed to Intense Noise

Group	Sex	Treatment	N	0 hrs Adrenals*	0 hrs Plasma**	3 hrs Adrenal	3 hrs Plasma	6 hrs Adrenal	6 hrs Plasma
I	M	Controls	9	3.0 ± 0.8	149 ± 9	4.7 ± 0.4	148 ± 4	3.0 ± 0.9	161 ± 10
II	M	Sound (114 db, 10-20 kc)	15	3.5 ± 0.6	146 ± 27	3.7 ± 0.5	146 ± 9	4.5 ± 0.7	150 ± 17
III	M	Controls	12	4.6 ± 1.6	63 ± 7	5.1 ± 0.7	62 ± 13	4.5 ± 0.9	50 ± 16
IV	M	Sound (149 db, 150-4800 cy/sec)	12	5.1 ± 1.2	69 ± 2	4.6 ± 1.4	59 ± 8	5.0 ± 1.8	45 ± 11
V	F	Controls	12	7.2 ± 1.9	49 ± 6	6.8 ± 0.4	49 ± 6	8.3 ± 1.8	54 ± 18
VI	F	Sound (149 db, 150-4800 cy/sec)	12	7.4 ± 0.7	66 ± 12	6.9 ± 1.0	72 ± 15	8.2 ± 1.0	49 ± 8

* mgm. cholesterol per 100 mgm. adrenal
** mgm. cholesterol per 100 ml. plasma

Additional data included taking adrenal weights and measuring serum electrolytes 0, 3 and 6 hours after noise exposure. Table 6 reveals that noise likewise has no effect on adrenal weight or serum electrolyte concentrations.

These results serve to confirm the major finding from previous experiments reported in Sections IV.2 and IV.3 that single noise exposure under the conditions specified, does not appear to act as a severe stressor since the pituitary-adrenal system is not activated to any appreciable extent.

D. Effects of Noise on Blood Glucose Levels

Purpose:

The purpose of this exploratory study was to determine the stress effects of exposure of rats, mice and guinea pigs to intense noise as measured by changes in blood glucose levels.

Procedure:

Two separate experiments were designed to measure the effects of intense noise on blood glucose levels. The Folin-Wu technique was used in each case for the analysis of the blood.

In the first experiment the same animals were used as were employed in the serum electrolyte and ascorbic acid studies (Section IV.3, A and B). The animals were exposed for fifteen minutes each day to noise at an intensity of 139-143 db and a frequency of 150-4800 cps.

Four groups of eighteen animals were selected from a colony of Swiss albino mice for the second study. The first group was deprived of food for twenty-three hours a day and exposed to intermittent noise. The second group was exposed to intermittent noise, but food was available at all times. The two control groups were not exposed to noise, the one group being deprived of food for twenty-three hours a day, the other having food provided ad libidum. Sound intensity and frequency were the same as employed in the first experiment.

Table 6. Adrenal Weights and Serum Ion Levels of Mice Exposed to Intense Noise (143 db, 150-4800 cy/sec)

Animal	Sex	N	(gms) Body wt.	hrs. after exposure	(mgms) adrenal	Na	meq/l Ca	K
Control	M	4	29 ± 3	0	31 ± 4	160 ± 2	4.8 ± 0.5	4.6 ± 0.4
Expr.	M	4	28 ± 1	0	34 ± 6	161 ± 9	4.8 ± 0.4	4.5 ± 0.7
Control	F	4	30 ± 6	0	55 ± 4	154 ± 2	4.2 ± 0.4	4.4 ± 0.5
Expr.	F	4	27 ± 1	0	53 ± 6	159 ± 3	4.7 ± 0.2	4.2 ± 0.1
Control	M	4	30 ± 2	3	32 ± 2	158 ± 4	4.6 ± 0.5	5.3 ± 1.0
Expr.	M	4	31 ± 2	3	31 ± 2	151 ± 8	4.6 ± 0.2	4.2 ± 0.4
Control	F	4	28 ± 1	3	61 ± 7	161 ± 4	4.8 ± 0.5	4.5 ± 0.4
Expr.	F	4	24 ± 4	3	60 ±11	159 ± 3	4.6 ± 0.2	4.5 ± 0.2
Control	M	4	28 ± 2	6	34 ± 6	153 ± 7	4.4 ± 0.1	3.8 ± 0.6
Expr.	M	4	29 ± 1	6	32 ± 7	148 ±11	4.3 ± 0.7	3.5 ± 0.4
Control	F	4	23 ± 3	6	57 ± 6	162 ± 0	4.5 ± 0.6	4.7 ± 0.4
Expr.	F	4	26 ± 1	6	57 ± 6	162 ± 0	4.8 ± 0.2	4.9 ± 0.7

Results:

The results of the first experiment appear in Table 7. As can be seen from the results obtained there were no significant differences in the blood sugar levels of the noise exposed animals and the controls.

Data obtained from the study in which food restriction was combined with intermittent noise also indicate that there were no significant differences in the blood sugar levels obtained in the experimental and the control groups. The results of this experiment appear in Table 8. It was concluded from these studies that the noise did not act as a sufficiently severe stressor to effect striking changes in glucose metabolism as far as could be determined from measures of blood glucose concentrations.

Table 7. Blood Sugar Levels in Animals Exposed to Intense Sound (139-143 db, 150-4800 cps)

Animal	N	Treatment	Duration of Experiment	Blood Sugar (mg % ± S. E.)
Rat				
male	6	Controls	28 days	92.9 ± 3.9
female	5	Controls	28 days	97.4 ± 1.4
male	5	Noise		
		15 min/day	28 days	96.2 ± 0.6
female	6		28 days	91.4 ± 6.6
Guinea pig				
male	6	Controls	29 days	108 ± 25
female	5	Controls	29 days	125 ± 30
male	6	Noise	29 days	135 ± 34
female	6	15 min/day	29 days	102 ± 26
	23			117 ± 10
Mice				
male	4	Controls	30 days	116 ± 10
female	6	Controls	30 days	107 ± 23
male	6	Noise	30 days	99 ± 5
female	5	15 min/day	30 days	126 ± 21
	21			111 ± 3

Table 8. Blood Sugar Levels in Mice Exposed to Intermittent Noise and Food Restriction

Sex	N	Treatment	Blood Sugar (mg % ± S. E.)
Male	10	No sound Food ad libitum	113 ± 4.4
Female	6	No sound Food ad libitum	117 ± 3.4
Male	8	Intermittent Sound Food ad libitum	114 ± 5.9
Female	6	Intermittent Sound Food ad libitum	110 ± 3.4
Male	2	No sound Food Restricted	84 ± 5.3
Male	2	Intermittent Sound Food Restricted	84 ± 1.4

IV.4. EFFECTS OF NOISE ON WEIGHTS OF ADRENALS AND OTHER ORGANS

As an adjunct to the studies reported in Sections IV.2 and IV.3 analyses were made of noise effects on adrenal weights and weights of target organs influenced by adrenal secretions. In the following studies the noise levels used were approximately 136-143 db in the 150-4800 cps frequency range.

Procedure:

The rats, guinea pigs and mice used for this work were the same as those used in studies of serum electrolyte, ascorbic acid and blood sugar changes following noise exposure. In the first three studies animals were exposed to intense, low frequency noise (ca 140 db, 150-4800 cps) fifteen minutes daily for a period of about four weeks. All animals were autopsied at this time and various organs removed and weighed on a torsion balance.

In the fourth study the same sound levels and frequencies were used but the noise was automatically regulated to permit alternate fifteen minute exposures and fifteen minute rest periods throughout the day for a duration of ten days.

A. Organ Weight Changes in Rats Exposed to Intense Noise.

Data obtained from organ weights of control rats and rats exposed to noise (140 db, 150-4800 cps) fifteen minutes daily for twenty-eight days are summarized in Table 9. No significant differences were found between organ weights of control and experimental rats irrespective of whether the data were analyzed on an actual weight basis or using organ weights corrected for body weight differences.

B. Organ Weight Changes in Guinea Pigs Exposed to Intense Noise.

Mean organ weight data and mean organ:body weight ratios from a similar study using guinea pigs are summarized in Table 10. Analyses of these data again revealed no differences between

Table 9. Weights of Organs Taken from Rats Exposed to Intense Noise (136-143 db in 150-4800 cps Freq. Band)

Treatment	N	Body Wt. (grams)	Testes	Prostate	Seminal Vesicles	Adrenals	Thymus	Spleen
			Mean Organ Weights (gms) ± Standard Error					
Controls (No Sound)	6 M	256±16	2.92±.05	0.56±.05	0.99±.06	.0293±.0023	.327±.029	1.46±.26
	6 F	190±16	----	----	----	.0416±.0024	.277±.031	0.84±.08
Noise 15 Min/Day For 28 Days	6 M	274±16	3.06±.12	0.59±.05	1.18±.08	.0290±.0011	.324±.060	0.81±.13
	6 F	203±14	----	----	----	.0462±.0016	.208±.027	0.51±.06
			Mean Organ Weights (grams per 100 gms body weight) ± S. E.					
Controls	6 M	256±16	1.16±.07	0.22±.02	0.39±.02	.0114±.0003	.129±.012	.564±.076
	6 F	190±16	----	----	----	.0220±.0011	.145±.014	.446±.044
Noise	6 M	274±16	1.13±.06	0.22±.01	0.43±.03	.0108±.0009	.111±.018	.289±.036
	6 F	203±14	----	----	----	.0232±.0016	.105±.015	.259±.036

Table 10. Weights of Organs from Guinea Pigs Exposed to Noise (136-143 db, 150-4800 cps)

Treatment	N	Body Wt. (grams)	Testes	Prostate	Seminal Vesicles	Adrenals	Spleen	Thyroids
			Mean Organ Weights (gms) ± Standard Error					
Controls (No Sound)	6 M	432±29	2.07±0.24	.447±.090	1.09±.25	.236±.012	0.87±.12	.0527±.0034
Controls (No Sound)	5 F	406±36	----	----	----	.232±.017	0.84±.08	.0562±.0027
Noise 15 Min/Day For 29 days	6 M	368±15	1.51±0.25	.347±.042	0.94±.24	.222±.006	0.81±.10	.0524±.0038
	6 F	410±22	----	----	----	.232±.010	0.76±.04	.0629±.0047
			Mean Organ:Body Weight Ratios (gms/100 gm body wt. ±S. E.					
Control	6 M	432±29	0.48±0.05	.107±.014	.238±.044	.0563±.0054	.202±.023	.0123±.0007
Control	5 F	406±36	----	----	----	.0581±.0045	.209±.017	.0141±.0008
Noise	6 M	368±15	0.41±0.07	.094±.010	.248±.065	.0608±.0022	.218±.024	.0144±.0012
Noise	6 F	410±22	----	----	----	.0570±.0027	.186±.016	.0154±.0013

organ weights of control and noise exposed guinea pigs. A slight increase in the adrenal weights of males was found but not in the females. It may be significant that the pattern of serum ascorbic acid changes reported in Section IV.3 follows the same trend as that suggested by the changes in relative adrenal weights.

C. Organ Weight Changes in Mice Exposed to Intense Noise.

Data obtained from organ weight analysis of mice at autopsy appear in Table 11. It can be seen from these results that, unlike the rats and guinea pigs, mice exposed to noise for fifteen minutes per day have heavier adrenals than controls. These changes were significant at the 1% level of confidence using Fisher's "t" test. Contrary to expectations, thymic and spleen involution did not occur; instead, the weights of these organs were heavier in experimentals than controls. It was concluded from this that although noise stimulation caused an hypertrophy of the adrenal, excessive activation of the adrenal by pituitary ACTH did not occur under the specified experimental conditions.

D. Organ Weight Changes in Mice Exposed to Intermittent Noise and Food Restriction.

In the fourth experiment mice were exposed to intermittent noise throughout each day and fed for only one hour during that period. Another group was exposed to intermittent noise but fed ad libitum. Two control groups, one fed ad libitum and the other fed one hour per day, were not exposed to noise.

Organ weight changes and records of gastric ulceration appear in Table 12. The results indicate that as in the previous study (Section IV.4) adrenal hypertrophy occurred with noise stimulation alone, but there was a less marked adrenal hypertrophy when noise stimulation was coupled with food restriction.

As one might expect, thymic involution was greater following noise and food restriction than with noise alone. Gastric ulceration was present in mice subjected to food restricted controls as well as in animals subjected to intermittent noise and food restriction, but was absent in animals exposed only to intermittent noise.

Table 11. Weights of Organs from Mice Exposed to Noise
(136-143 db, 150-4800 cps)

Treatment	N	Body Wt. (grams)	Testes	Seminal Vesicles	Adrenals	Thymus	Spleen
			Mean Organ Weights ± Standard Error				
Controls (No Sound)	5 M	26.7±1.0	0.17±.01	0.12±.01	.0032±.0002	.0111±.0010	.140±.017
	6 F	23.6±0.7	----	----	.0046±.0003	.0196±.0017	.105±.012
Noise (15 Min/Day for 30 days)	6M	23.5±0.9	0.17±.01	0.15±.01	.0033±.0001	.0163±.0008*	.170±.016
	6 F	22.6±0.6	----	----	.0056±.0002*	.0147±.0021	.131±.010*
			Mean Organ Weights (gms per 100 gms body wt.) ± S. E.				
Controls	5 M	26.7±1.0	0.62±.04	0.46±.02	.0113±.0009	.0411±.0025	.522±.062
	6 F	23.6 ± 0.7	----	----	.0196±.0011	.0833±.0073	.440±.036
Noise (15 Min/Day for 30 days)	6M	23.5±0.9	0.72±.01	0.62±.03*	.0141±.0004*	.0698±.0034*	.734±.082
	6 F	22.6±0.6	----	----	.0246±.0007*	.0645±.0082	.581±.045

*Significant change at the 1% level of confidence using Fisher's "t" test.

Table 12. Adrenal and Thymus Weight Changes with Intermittent Noise and Food Restriction

Sex	N	Treatment	Adrenal wt (mg ± S. E.)	Thymus wt (mg ± S. E.)	Gastric Ulcers
Male	12	Control	37 ± 1	165 ± 26	Absent
Male	15	Food Restriction	39 ± 2	63 ± 14	Present
Male	10	Intermittent Noise	51 ± 3	96 ± 11	Absent
Male	10	Intermittent Noise; Food Restriction	42 ± 2	40 ± 4	Present
Female	6	Control	55 ± 3	327 ± 62	Absent
Female	10	Food Restriction	50 ± 3	131 ± 108	Present
Female	6	Intermittent Noise	68 ± 5	219 ± 91	Absent
Female	5	Intermittent Noise; Food Restriction	60 ± 5	65 ± 7	Present

It was concluded on the basis of these data that noise stimulation acted as a mild stressor in mice since it caused adrenal hypertrophy but had no changes in target organs. Food restriction, on the other hand, represents a severe stress which can be aggravated when combined with the stress of noise.

IV.5. EFFECTS OF NOISE ON BEHAVIOR

The purpose of these studies was to determine what behavioral responses occur in animals exposed to intense noise situations. It is fairly obvious from the foregoing sections (dealing with physiological effects of noise) that animals can successfully cope with short periods of daily exposure to intense noise. It was desirable to determine whether adaptive behavioral responses might provide a partial explanation of why noise does not act as a harmful physiological stress to animals.

Procedure:

The sound apparatus used in this study consisted of a white noise generator, Bogen amplifier, Navy Beach amplifier and a B-24 University loudspeaker. During exposure to noise animals were placed in a reverberant chamber which contained six separate cages. Twelve male and twelve female Jax A mice were used in this study. Twelve of the mice were used as experimentals and twelve as controls. Mice were placed in the reverberant chamber in groups of six with three males and three females in each group. The sexes were kept separate outside of the experimental situation. Groups of Wistar rats and of Hartley strain guinea pigs were set up in a similar manner.

The behavior of the animals was observed for fifteen minutes before the noise was applied, the animals in each cage being observed for five minute periods. The animals were then exposed to noise at a level of 139-143 db (re 0.0002 dynes/cm^2) and a frequency of 150-4800 cps for fifteen minutes. Behavior observations were made during the period of noise stimulation and for fifteen minutes after the noise was turned off.

Behavior observations were made on special forms to facilitate rapid recording. A copy of a data sheet with typical recordings appears in Fig. 20. Animals were identified by picric acid marks on the back fur and the position of each animals was recorded by placing the number of the animal in one of the small squares inside the thirty second interval blocks on the data sheet. Each square in the blocks represented a unit of space in the cage of 3 and 1/3 inches by 3 and 1/3 inches. The distance traveled by each animal in a five minute period was measured by placing a piece of tracing paper over the data sheet and plotting in one block the positions recorded in each of the thirty second intervals for a given animal. The points were then joined and the resulting lines were measured with a special rule calibrated in terms of the units or small squares on the data sheet. A sample tracing of the movements of the animals recorded in Fig. 20 may be found in Fig. 21. Recordings of the various types of activity observed were made by placing a code letter under the animal's number in the rectangular block at the lower right hand corner of the data sheet. Descriptions of these activities follow in the results.

Results:

To facilitate discussion the types of behavior observed in the animals in this study have been arbitrarily grouped into three categories: exploratory activity, social activities and individual activities. The results include the effects of intense noise stimulation on each of these types of behavior. The results as presented in this section have been gleaned from detailed analysis of the data obtained from the mice. The data gathered from the rats and guinea pigs showed the same trends as that gathered from the mice. Where the rat and guinea pig data indicated slight differences from that obtained from the mice it was analyzed in more detail and these results are also included in this section.

A. Effects of Intense Noise Stimulation on Exploratory Activity.

Exploratory activity refers to the type of activity the mice engage in shortly after introduction to the cage. The mice move with their bodies close to the ground, usually following

DATE 11/22/55

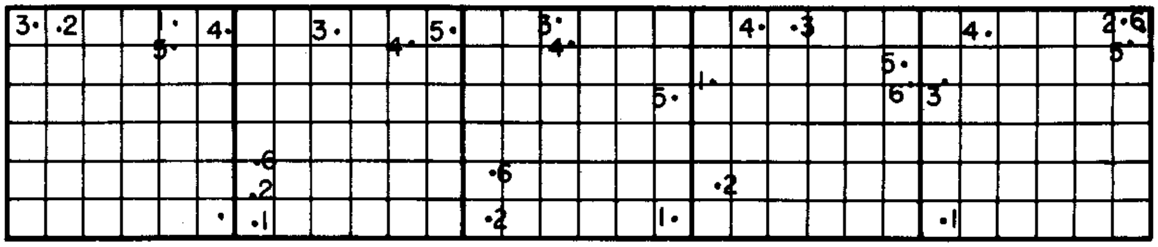
ANIMALS: MICE   ROOM TEMP. 24°C

GROUP: EXPERIMENTAL

NUMBERS 1–3 MALES

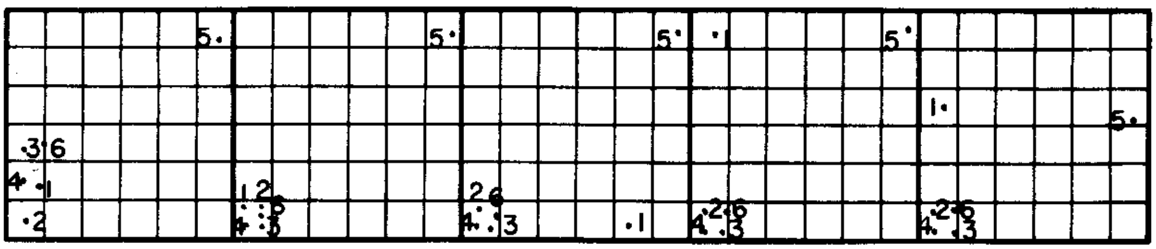
NUMBERS 4–6 FEMALES

PERIOD BEFORE NOISE



1 Large block = one 30 sec. interval   1 Small block = 1 distance unit (equivalent to $3\frac{1}{3}$ inches)

PERIOD DURING NOISE



ANIMAL INITIATING CONTACT

BEFORE NOISE

Mouse Number (Animal receiving contact)	1	2	3	4	5	6
1						
2	C	W Sc				G
3			W			
4			G	W		
5					W	
6						

DURING NOISE

	1	2	3	4	5	6
1						
2	G	W G		C		
3						
4	G C		G			
5	C				W W	W
6						Sc Sc

Sc = Scratching   G = Genital smell

W = Washing   C = Contact c̄ bite

B = Bite   N = Nose contact

FIG. 20, DATA SHEET SHOWING METHOD OF RECORDING EXPLORATORY ACTIVITY AND SOCIAL CONTACTS BEFORE AND DURING EXPOSURE TO NOISE

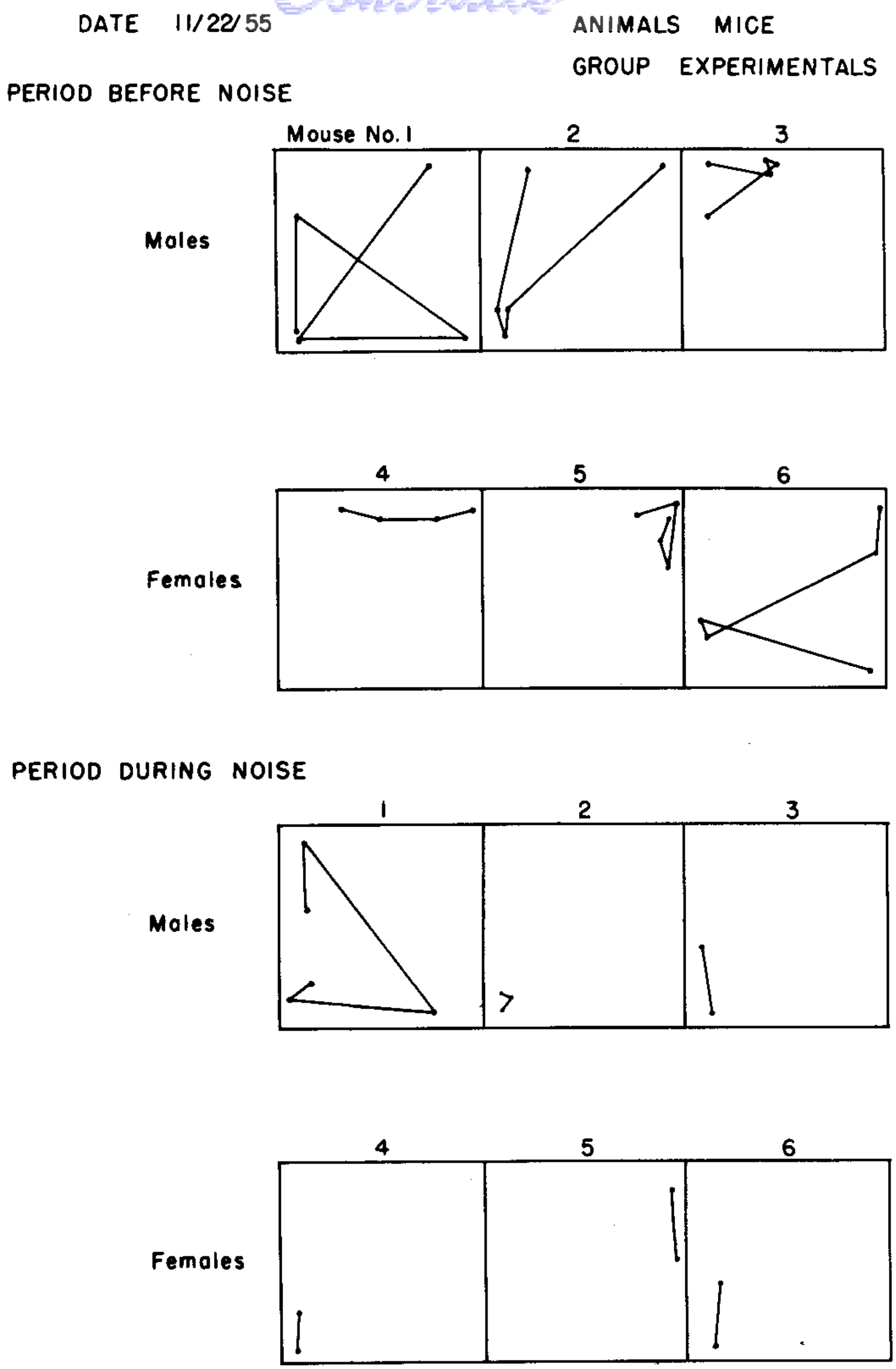


FIG. 21, TRACINGS OF DATA IN FIGURE 20 SHOWING DISTANCE TRAVELED BY INDIVIDUAL MICE DURING $2\frac{1}{2}$ MINUTE PERIODS the walls of the cage and occasionally stopping to rise on their haunches and sniff about in the air. Occasionally the mice dart from one side of the cage to the other, generally following a diagonal path from one corner of the cage to another. Upon reaching the corner of the cage the mice sniff about before continuing on. Very frequently the mice poke their noses through the wire grid floor or through the air holes in the lucite panel. Contacts with other animals are often made during the exploratory activities and these will be considered separately below.

Although exploratory activity includes all the various activities enumerated above, for comparative purposes total distance covered per five to fifteen minute observation period was chosen as the most reliable measure of exploratory activity. The mean activity during a five minute observation period for individual noise exposed mice was computed and a sample of these calculations appears in Fig. 22. The letters B, D, and A in this graph and the following ones refer to an average amount of a particular activity per unit time before, during and after noise exposure respectively.

Fig. 22 indicates that a noticeable reduction in activity occurs following exposure of mice to noise. In most cases activity during noise exposure as contrasted with pre-noise levels was lowest in the third and fourth weeks of the experiment. Male mice showed an overall increase in levels of exploratory activity in each of the observations periods during the last two weeks of the experiment. In female mice there was a relative decrease in the overall levels of exploratory activity in each of the observation periods during the second and third weeks of the experiment. The mice generally showed a return to pre-noise levels of activity in the post-noise period of observation. In the guinea pigs a greater number of animals showed a decreased level of activity in the post-noise period of observation as contrasted with the period of noise stimulation.

In summary it can be stated that noise exposure resulted in decreased activity in all of the animal groups used during the period of stimulation. These changes in the levels of exploratory activity were due to both group and individual responses.

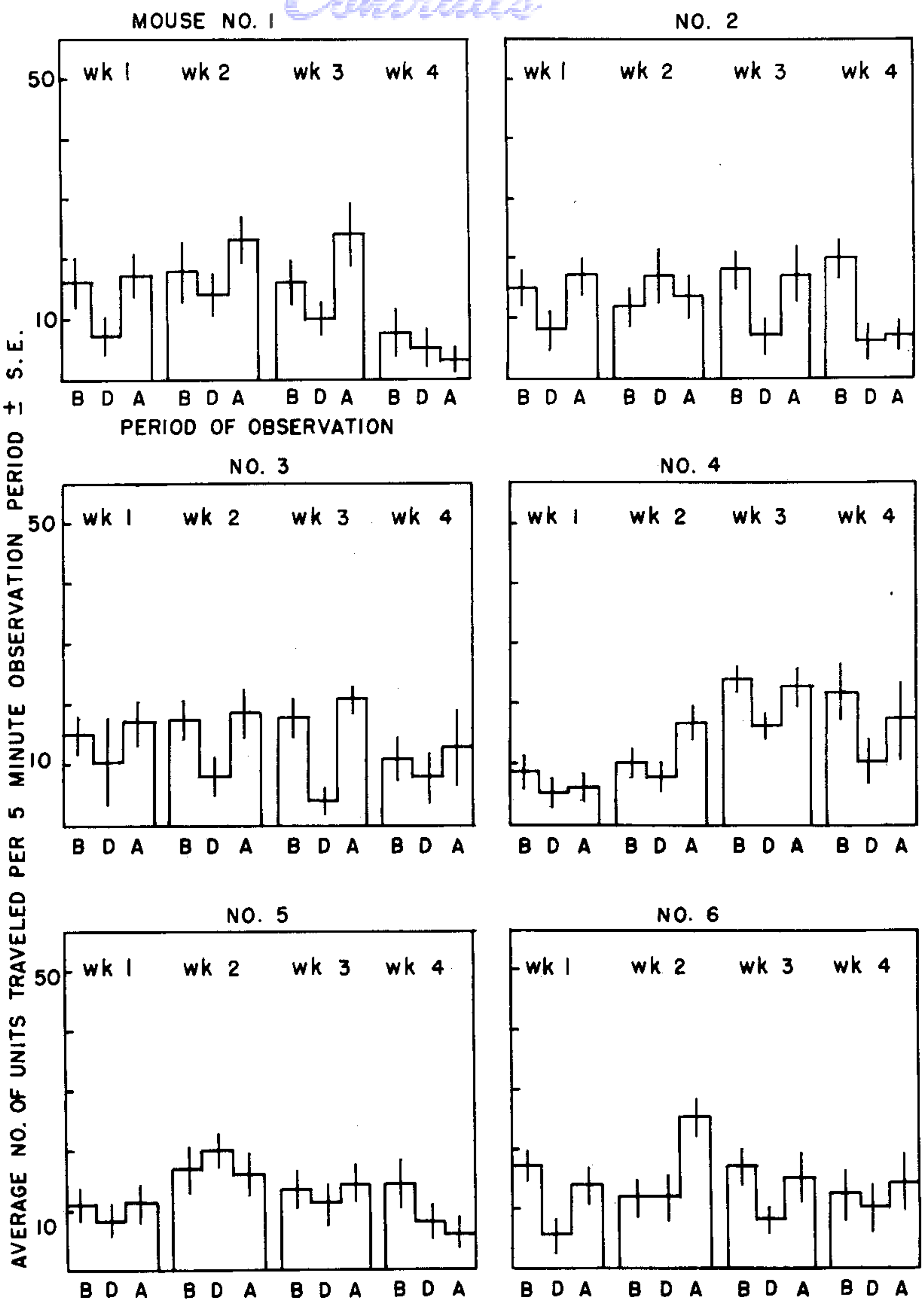


FIG. 22, LEVELS OF EXPLORATORY ACTIVITY IN NOISE EXPOSED MALE MICE

1. Responses of the group.

Two types of group responses were observed in the animals throughout the course of the experiment: huddling into very compact groups and loose aggregations of mice. To facilitate discussion these responses will be referred to as huddling and aggregation in the remainder of the report.

Huddling is characterized by the animals crowding into a closely knit group with animals piling on top of one another and having their noses directed toward a cage corner. Little or no movement was observed among the animals while they were huddled. Huddling was a characteristic response of the animals during noise stimulation and may be considered as one of the factors contributing to the decreased levels of exploratory activity during noise exposure. During the latter part of the experiment there was a slight tendency for huddling to be less frequent. Fig. 23 depicts huddling in mice and guinea pigs and Fig. 24 shows rats in the huddled position.

Aggregation is characterized by the animals coming together in a loose group in the vicinity of the cage corner. In such a grouping piling of animals on top of one another does not occur and animals leave the group from time to time to move about the cage. Aggregation was observed in the pre-noise period, first becoming apparent during the latter part of the first week of the experiment. Although aggregation was absent on some days there was an increased tendency toward this type of grouping as the experiment progressed. The aggregation generally became a huddle when the noise was turned on.

The major effect of noise stimulation on group responses appeared to be an increased tendency on the part of the mice to form a loosely organized group in the pre-noise period which was followed by the formation of a compact group when the noise was turned on.

2. Responses of individuals.

The responses of individuals during noise exposure consisted in animals being caught away from the group in the "freezing" position or in animals going to and from the group at will.

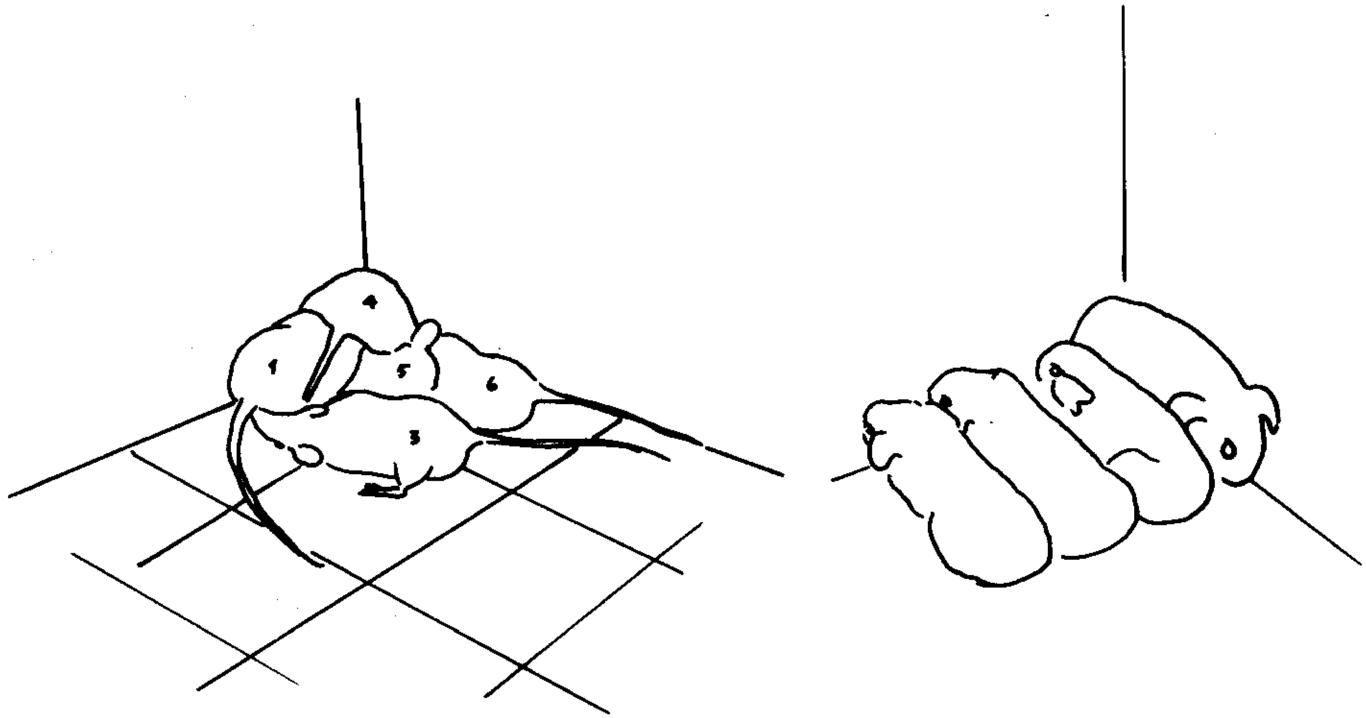


FIG.23, HUDDLING RESPONSE OF MICE AND GUINEA PIGS IN CORNER DURING NOISE EXPOSURE.

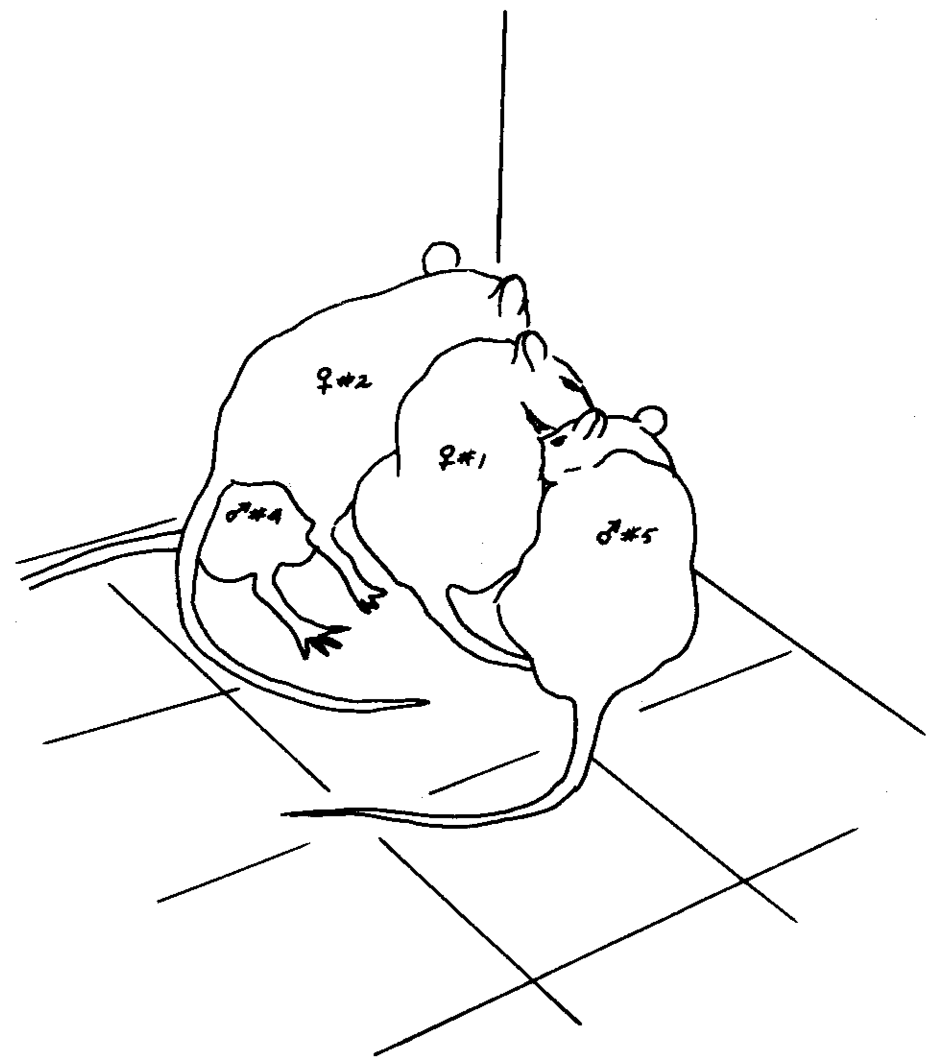


FIG.24, HUDDLING RESPONSE OF RATS DURING NOISE EXPOSURE.

Freezing is characterized by the animal coming to a dead halt upon exposure to noise, remaining in a crouched position with ears lowered and breathing rapidly. This response occurred during noise stimulation, often persisting in the post-noise period.

During noise exposure most of the animals generally remained huddled or in the freezing position, but occasionally a single animal or several animals would leave the huddle for a short time, dart rapidly about the cage and return to the huddled group. The huddle generally disintegrated when the sound was turned off, but some individuals very often remained motionless in the corner for the duration of the post-noise observation period.

B. Effects of Noise on Social Activities.

Social activities encompass those activities which involve the interactions of two or more animals and include aggressive biting, pursuit of one animal by another, genital smelling, body contacts not involving biting, sexual behavior and nose contacts. Nose contacts are characterized by one animal touching its nose to the nose of another.

Genital smelling and nose contacts were quite frequent, but general body contacts were observed most frequently. Aggressive behavior and mating behavior were not observed in the animals during the course of the experiment. In compiling the observations of the activities observed, all of the activities were grouped under the general heading of contacts. From data of daily observations of these contacts the mean number of contacts received and the mean number of contacts given by each animal within a sixty minute period were calculated for each week and for each of the observation periods. Graphs of these calculations appear in Figs. 25 and 26.

Data in Fig. 26 indicate that there was generally a decrease in the number of contacts given during the period of noise stimulation. In the last three weeks of the experiment there were increases in the frequency and the individual variability of the total number of contacts given resulting from decreased huddling and increases in exploratory activity on the part of

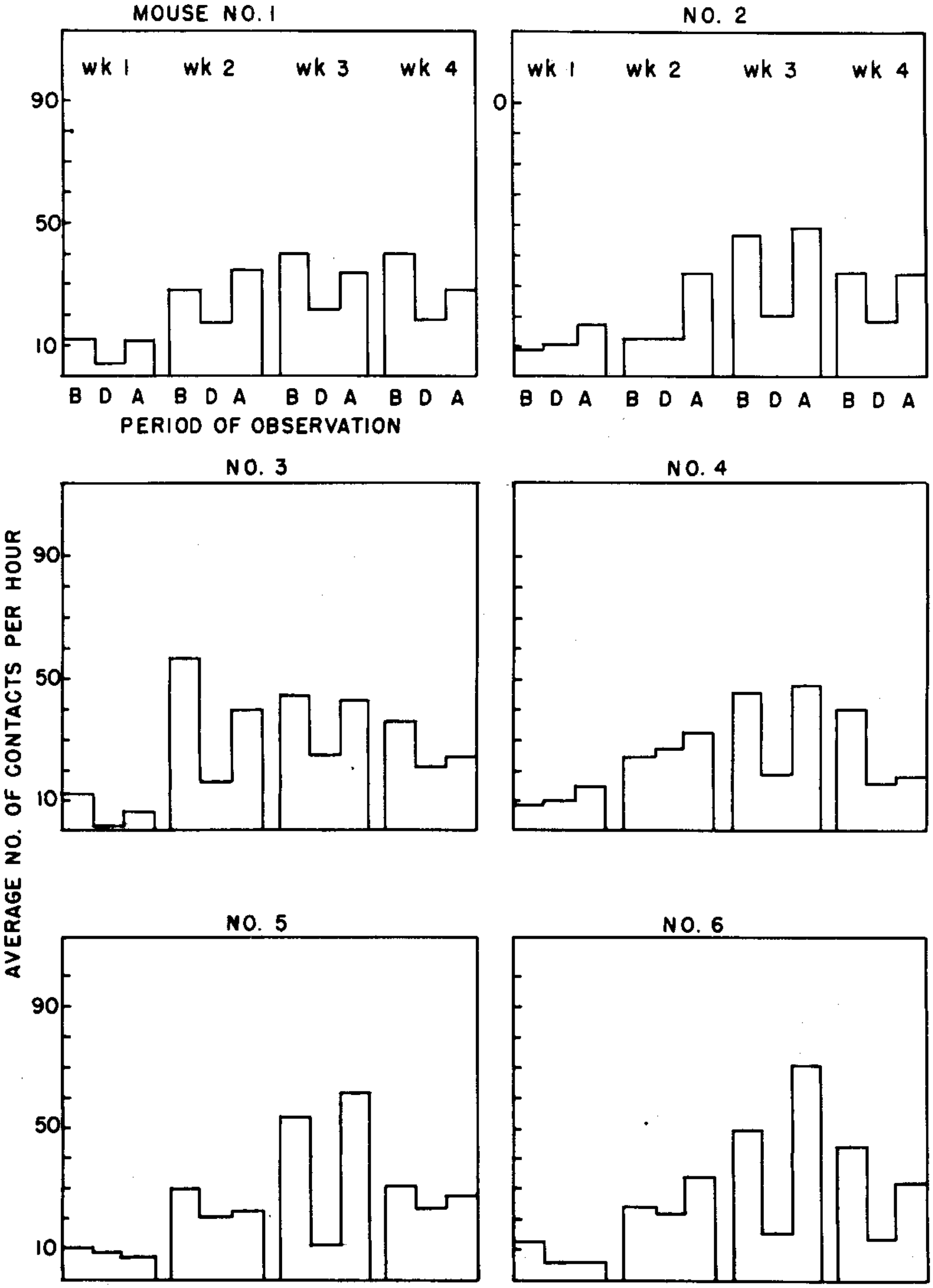


FIG. 25 INCIDENCE OF CONTACTS RECEIVED IN NOISE EXPOSED MALE MICE

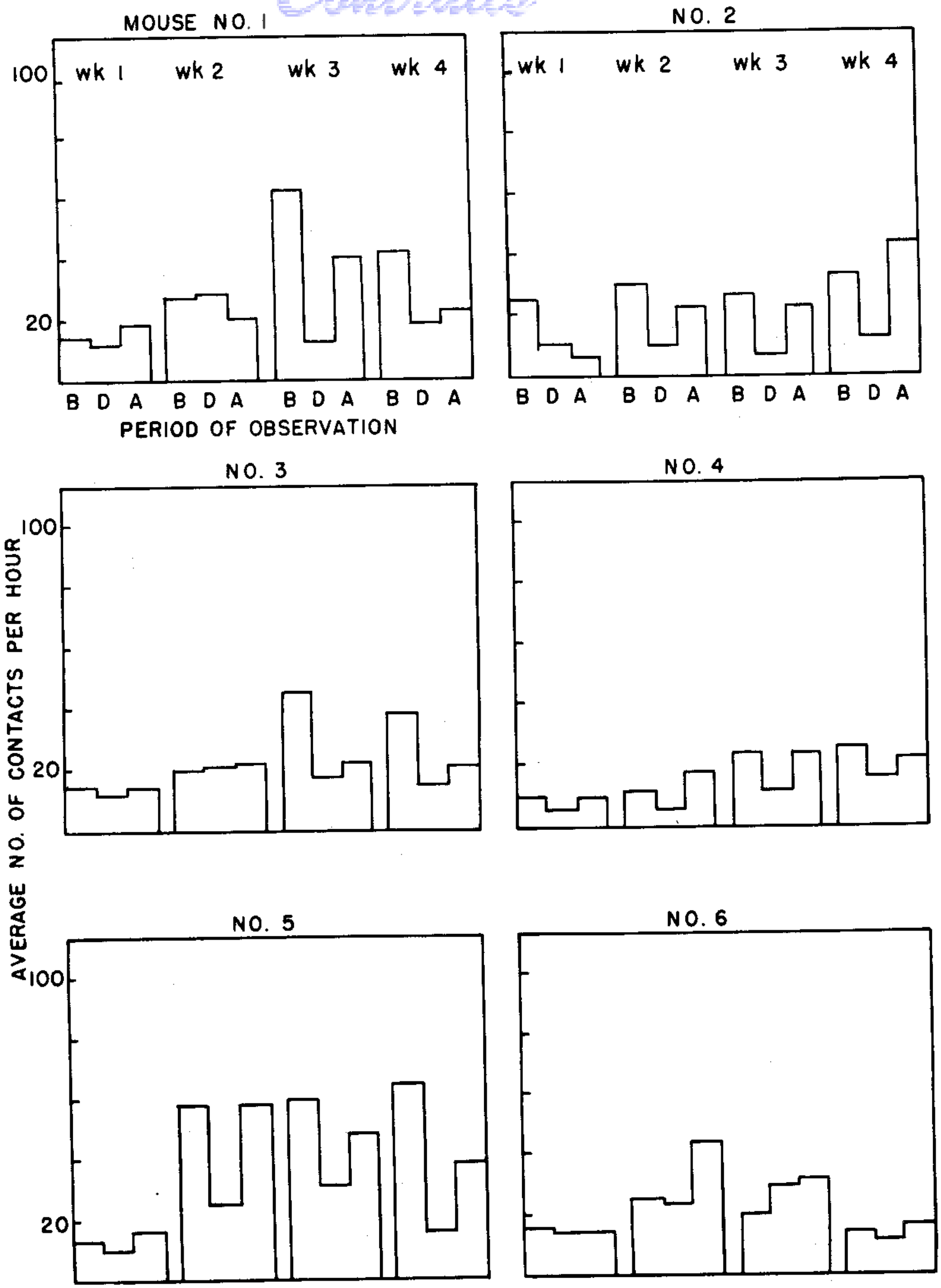


FIG. 26, INCIDENCE OF CONTACTS GIVEN IN NOISE EXPOSED FEMALE MICE some individuals. The data in Fig. 25 indicate that the same trends were evident in the contacts received by individual animals as were reflected in the results of exploratory activity and contacts given.

In summary it may be observed that noise stimulation resulted in a decreased number of contacts among the animals during noise exposure. Numbers of contacts made by the animals in the pre- and post-noise periods approximated the levels observed in the controls for the time of day at which the experimentals were observed.

C. Effects of Noise Stimulation on Individual Activities.

Individual activities consisted of washing and scratching. Washing is characterized by the animal licking its body or forepaws and rubbing its body and nose with its forepaws. Scratching, as observed in the rats and mice, is characterized by rapid rubbing of the forepaws at some point on the body, usually near the head region and around the ears. Attitudes assumed by mice and rats during washing and scratching are depicted in Figs. 27 and 28.

From daily observations of washing and scratching the mean number of times these activities occurred within an hour was calculated for each individual noise-exposed animal during each week in each of the observation periods. Graphs of typical data from observations of washing and scratching in noise exposed mice appear in Figs. 29 and 30.

In about half of the cases there was an increase in the frequency of washing and scratching during noise stimulation as contrasted with pre-noise levels. There was generally a marked increase of washing and scratching in the experimentals during noise stimulation as contrasted with the levels of these activities observed in the controls.

The overall results of the behavior observations indicate that a decrease in general exploratory activity occurs during noise exposure. There is also an increase in the individual variability of both group and individual responses following repeated stimulations with intense noise.

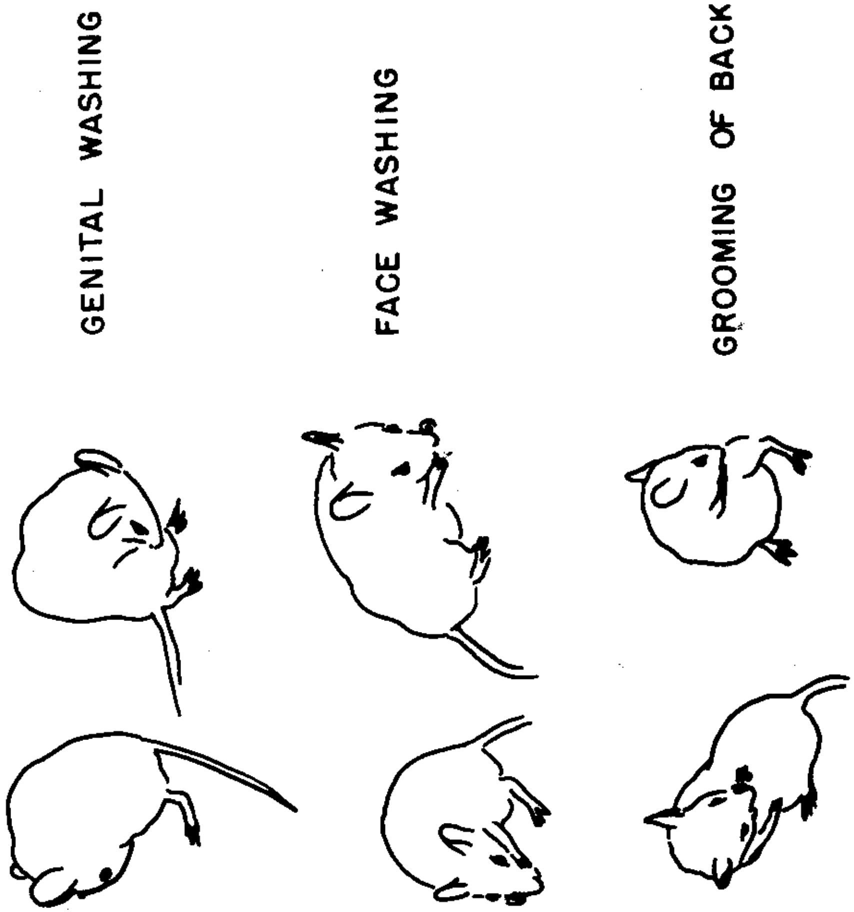


FIG. 27, WASHING AND GROOMING OF MICE DURING NOISE EXPOSURE

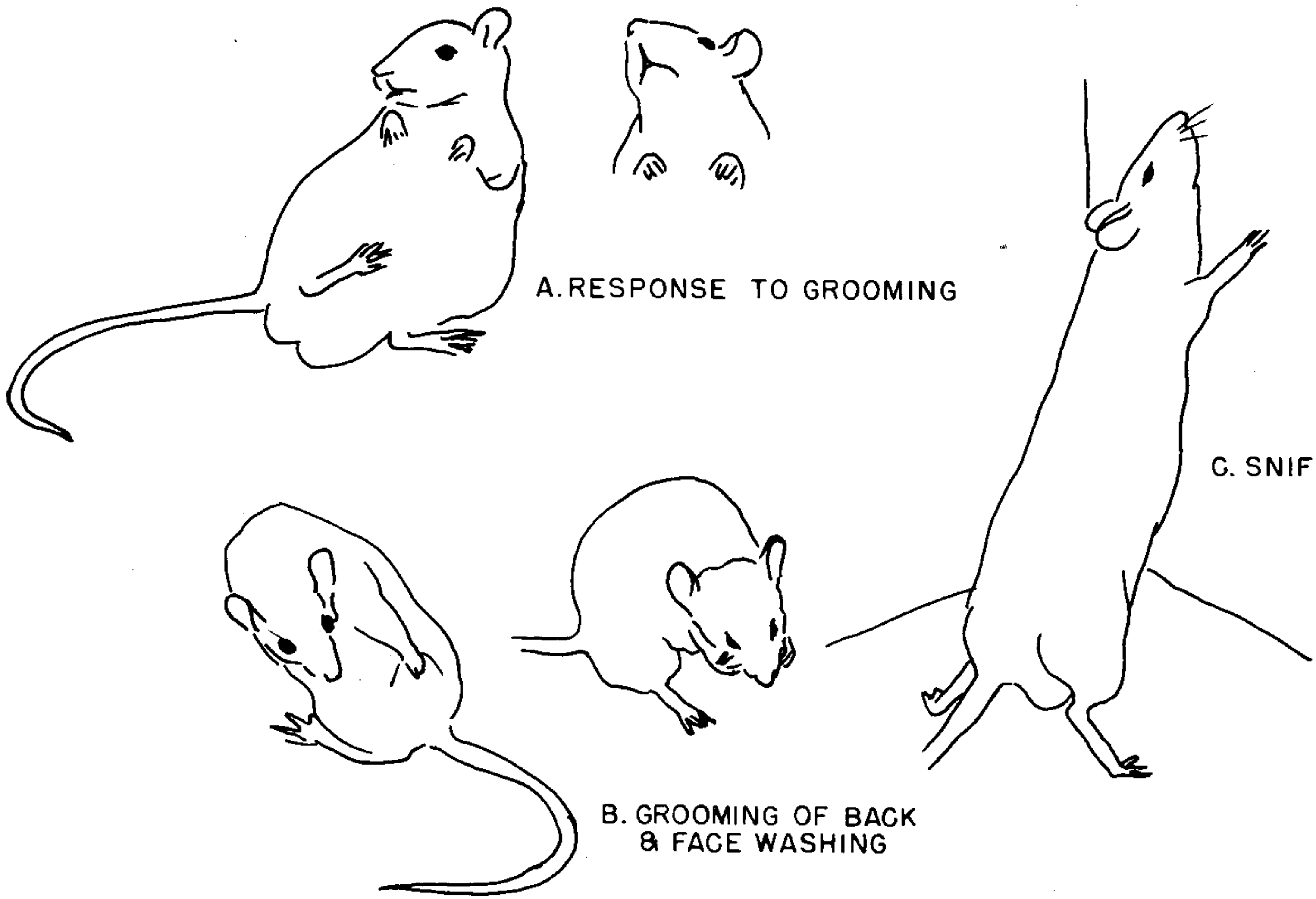


FIG. 28, TYPICAL ACTIVITIES OF RATS DURING NOISE EXPOSURE

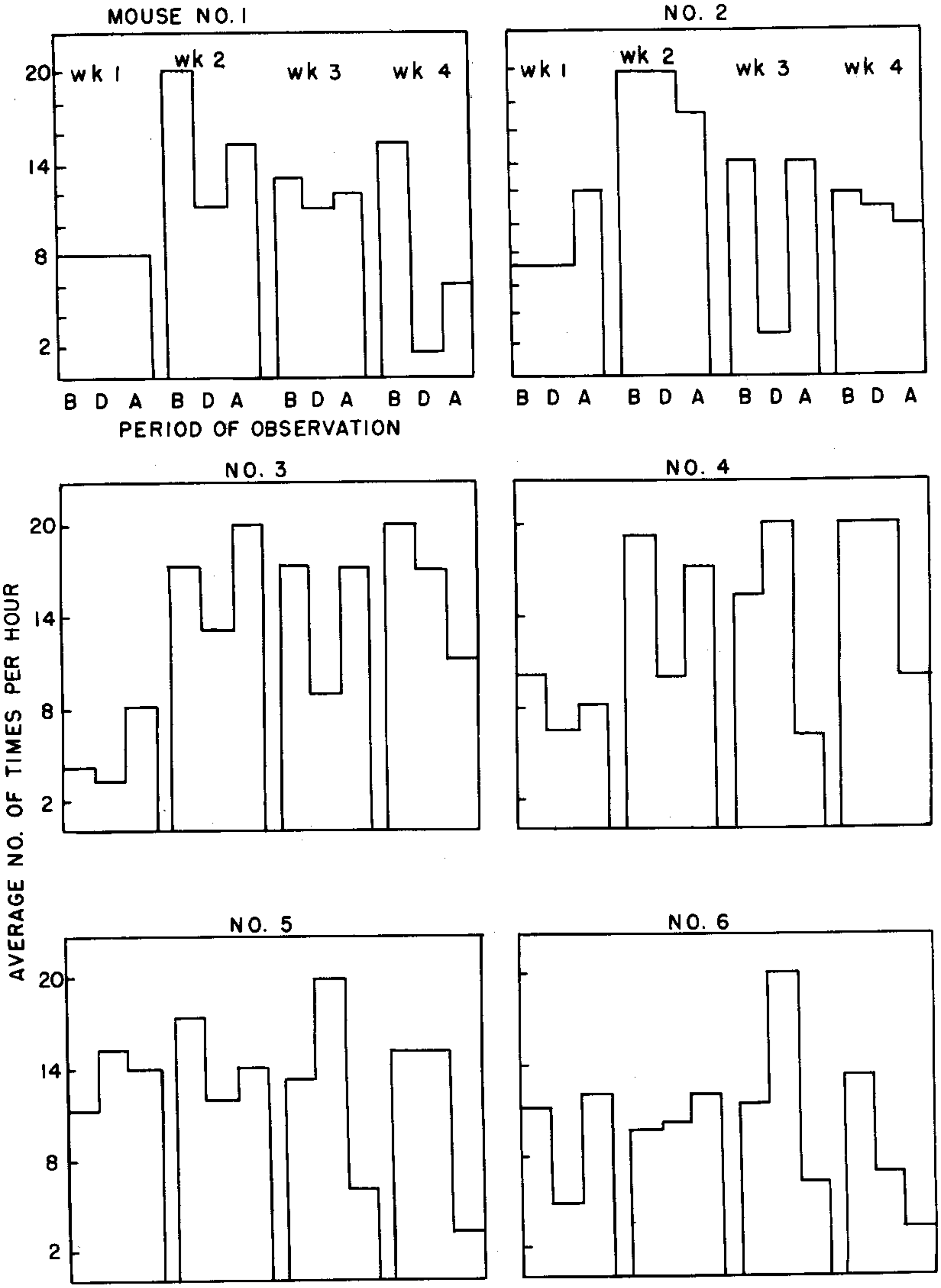


FIG. 29, INCIDENCE OF WASHING IN NOISE EXPOSED MALE MICE.

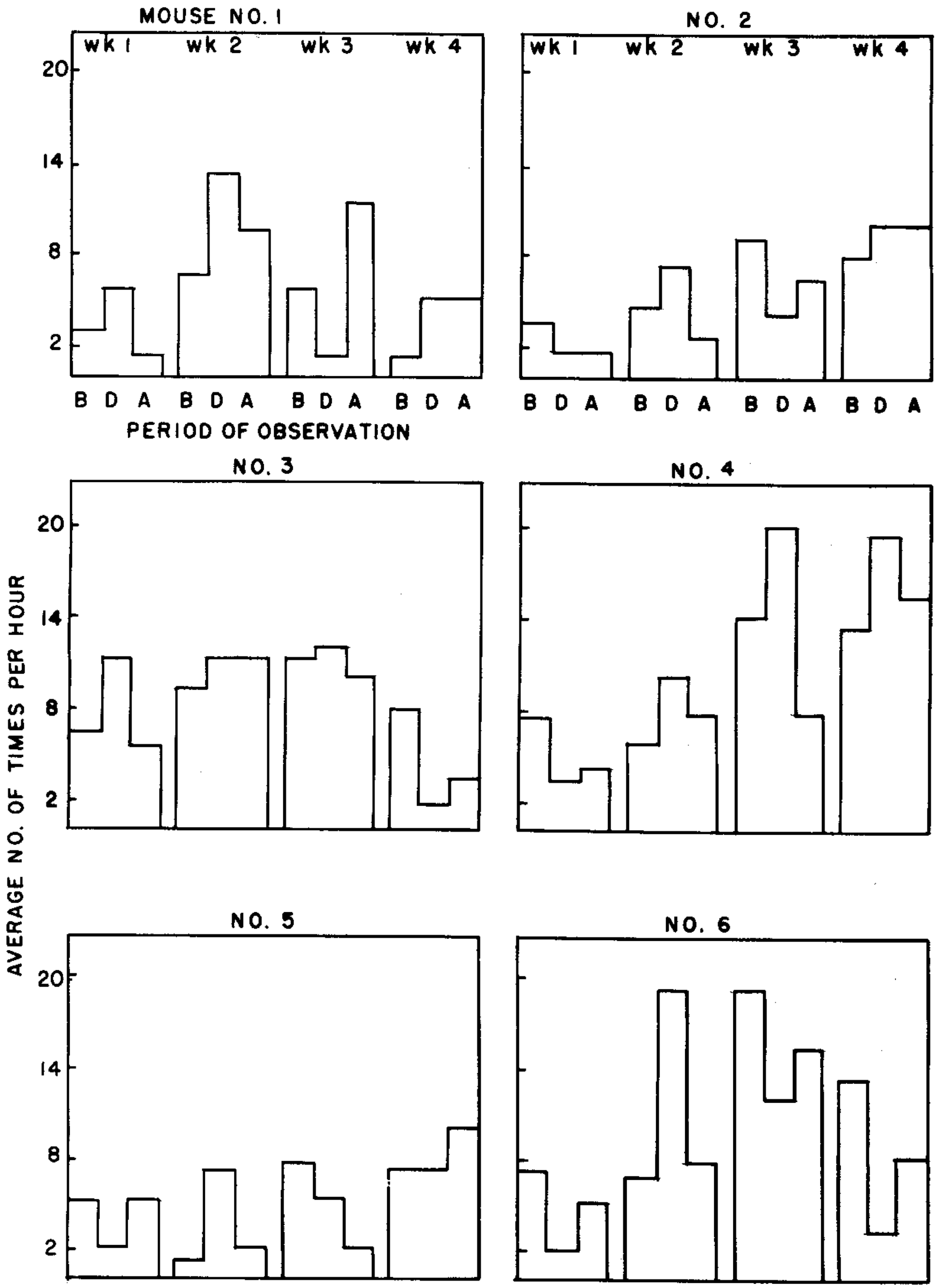


FIG. 30, INCIDENCE OF SCRATCHING IN NOISE EXPOSED FEMALE MICE

D. Summary of Behavior Studies.

The present study was designed to determine whether or not intense noise stimulation acts as a non-specific stress in animals. The particular approach in this study included the detailed analysis of behavior changes occurring in mice as a result of exposure to intense noise with supplementary material on behavior changes in rats and guinea pigs exposed to intense noise. In the following discussion an attempt will be made to evaluate the observed changes in behavior.

From data obtained in the present study it was concluded that the intense noise stimulation at low frequencies acted as a mild stress agent in mice, rats and guinea pigs.

The most striking effect of the intense noise stimulation on the animals was the reduction in the levels of both individual and social activities of the animals during the period of noise stimulation. The decreases in levels of activity were characterized by huddling and freezing of the animals during noise stimulation. Both huddling and freezing may be regarded as deviations from normal patterns of behavior.

The freezing response may have been indicative of a "fear" response to the sudden burst of sound and affords some indication that the animals were being stressed to some degree by the intense noise. The rapid respiratory rate which occurred during freezing may also be an indication that the animals were in an agitated condition.

Huddling is suggestive of a type of protective withdrawal of the animals from the source of the stimulation similar to "fear" responses described by others. The huddling response has obvious adaptive value since individuals are partially insulated from noise by other members of the group.

The aggregation may be regarded as an anticipatory type of behavior on the part of the animals preparatory to huddling and is indicative of conditioning to the experimental situation. The increased tendency of the animals to aggregate during the pre-noise period is another indication that the animals adapted to the noise.

The increased tendency toward aggregation, huddling, freezing and various escape attempts such as poking of noses through the grid cage floor and biting of the wire grid floor may be regarded as indicative of a state of anxiety in the animals exposed to noise. The manifestations of a state of anxiety which the behavior deviations observed in this experiment afford may be regarded as further evidence that the animals displayed adaptive behavior to the stress of the sound.

The higher incidence of washing and scratching in the animals during noise exposure and the higher incidence of these activities in experimentals as contrasted with the controls affords further evidence that the noise stimulation represented a stressful situation. Experimentals were also observed to engage in considerably more nose rubbing than the controls. Such activities were interpreted by Lloyd (1957) as "substitute" or "displacement" activities. Substitute activities have been described as irrelevant movements which occur when animals are under the influence of a powerful urge, but are unable to express this urge in an appropriate manner. These activities could possibly reflect an animal's inability to escape the noise situation and thus could be considered as another manifestation of anxiety. A more logical interpretation for the increases in nose rubbing may be that the nasal passages were irritated due to resonance of the sound in the nasal passages.

It may be concluded that intense noise acts as a mild stress agent as far as the behavioral response of animals is concerned. Evidence of this included freezing of individual animals, an increased tendency for animals to aggregate, huddling of animals and other behavioral expressions which have been arbitrarily designated as an "anxiety state". It is significant that the behavioral responses to noise of all three animal species used are characterized by a reduced amount of physical exertion and consequently a decreased expenditure of energy. It is more than likely that this has the effect of increasing the overall resistance of an animal to a stressful situation.

IV. 6. STUDIES USING INTENSE HIGH FREQUENCY NOISE

Most of the experiments discussed in preceding sections have dealt with intense noise using relatively low frequencies. One of the reasons for this is that conventional diaphragm type speakers are not capable of transmitting high frequencies at high sound pressure levels. As a consequence considerable effort and time were expended in the construction of a corona-type loudspeaker for use in studies of stress effects of intense high frequency noise.

The present section of this report summarizes the results of several exploratory studies using the 1.3 kw model of the corona-type loudspeaker system described in Section VII.

Procedure:

Most of the experimental work using the ionophone was performed on Swiss albino mice selectively bred for audiogenic-seizure susceptibility and seizure-resistance. These strains have been described in earlier papers by Frings (1952, 1953, 1956). Intensity levels employed were approximately 132 db re 0.0002 dynes/cm^2 in the 1-40 kc frequency band. No attempt was made to provide the reverberant exposure chamber with supplementary ventilation in the first series of studies since the chamber was provided with sufficient air holes to adequately maintain several dozen mice under normal conditions. Later it was found necessary to use ventilation since the ozone produced by the corona discharge reached lethal concentrations in the animal test chamber after only a short running time of the ionophone. The results of these studies are considered separately below.

Only single short exposures (one to six minutes duration) were used in the following experiments.

A. Early Noise Studies Using the Corona-type Loudspeaker System (Exposure Chamber Not Ventilated).

Several dozen experiments were run on approximately one hundred mice. Since the presence of ozone precludes drawing any definite conclusions regarding the stress effects of noise, only the general results of these studies are listed below:

(1) Atypical behavioral reactions occurred in mice during noise exposure. These included increased overall excitability, spasmodic jumping and changes in the respiration rate (irregular breathing and gasping).

(2) A marked increase in the incidence of convulsions in mice that were established as seizure-resistant through tests using low intensity high frequency noise (110 db, 10-20 kc). These mice also proved seizure-resistant when tested with high intensity low frequency noise (140 db, 150-4800 cps).

(3) An increase in the number of fatal convulsions in "seizure-resistant" mice during exposure to the noise produced by the corona loudspeaker system.

(4) A marked increase in fatalities in mice some time after the noise exposure. In the latter case some mice died within one to twelve hours after noise exposure even though no convulsions occurred during noise stimulation. Exposure times in these cases ranged from a five to six minutes duration.

The peculiar symptomatology of the mice which died following noise exposure led to the suspicion that the fatalities were due to ozone intoxication rather than noise stimulation per se. Analyses of the ozone concentration in the reverberant chamber proved this to be correct. The ozone levels in the test chamber after running the ionophone for fifteen minutes were ten to twenty fold greater than the toxic level listed for humans. A blower directed from the reverberant chamber toward a window in the acoustics laboratory was added to the sound apparatus. Ozone analyses with the ventilating system in operation showed no detectable amounts of ozone present in the reverberant chamber with the ionophone running continuously for a half hour.

In the following experiments there were no cases found of irregular breathing followed by death due to respiratory failure although the other symptoms of increased excitability, spasmodic jumping and increased seizure incidence remained the same.

B. Studies Using Modified Ionophone System with Ventilated Reverberant Chamber.

Procedure:

The noise levels used were about 132 db re 0.0002 dynes/cm^2 in the 2-40 kc frequency range. The exposure time was six minutes. Two types of studies were conducted, eosinophil response of mice to single noise bursts and the effects of noise on the incidence of convulsions. The eosinophil studies have been discussed earlier in Section IV.2

The approach used in investigating the effects of intense high frequency noise on the incidence of convulsions in seizure-resistant mice was as follows. Mice were selected at random from a laboratory stock of seizure-resistant mice. These were then tested for seizure susceptibility using a noise level of 110 db at 10-20 kc frequency. One group of mice was then exposed to high intensity, low frequency noise (139 db, 150-4800 cps) using the University B-24 loudspeaker system. A second group was exposed to high intensity, high frequency noise (132 db, 2-40 kc) using the 1.3 kw corona loudspeaker system. All animals were then retested with the 110 db, 10-20 kc noise stimulus. Records were kept on the incidence of convulsions and the type of convulsions which occurred. Individual mice having running seizures, clonic spasms or clonic-tonic seizures were marked with picric acid so that their future responses on retesting could be followed.

Results:

This study is still in progress and the data have not been analyzed in detail so only the major findings are summarized below. These are based on tests made using more than one hundred seizure-resistant mice.

(1) It was found that the incidence of seizures obtained using low intensity high frequency sound (110 db, 10-20 kc) was about two to five per cent.

(2) The same strain of mice exposed to intense noise at low frequencies (using University B-24 speaker system - 139 db, 150-4800 cps) has an incidence of about fifteen per cent. Retesting the mice with low intensity sound revealed a lower seizure incidence indicating they were not made more "seizure-prone".

(3) Exposure of another group of this strain of mice to the ionophone noise field (132 db, 1-40 kc) resulted in thirty to forty per cent seizures. Retesting mice with low intensity noise gave higher percentages of seizure than were had before stimulation with intense high frequency noise. The latter result suggests a possible sensitizing effect of intense high frequency noise.

(4) It was further observed that of the mice having seizures the number of clonic or clonic-tonic convulsions was higher in the group exposed to intense high frequency noise.

It was concluded from these data that intense noise is more effective in inducing convulsions in so-called seizure-resistant mouse strains at higher frequencies than at lower ones.

The data also suggest that audiogenic-seizure-susceptibility and seizure-resistance may have no basis other than a difference in the threshold of sensitivity to noise stimulation.

IV.7. DESIGN AND CONSTRUCTION OF CORONA LOUD-SPEAKER

It has long been recognized that an electric arc discharge in air will serve as a sound source. When such a discharge is interrupted or modulated at an audio rate, it is referred to as a "singing arc". Another type of electrical discharge, the radio frequency corona, can also serve as a sound source when modulated. Due to its low efficiency, a speaker of this type was not considered practical for many years. However, in 1954 Siegfried Klein developed a new corona type speaker which he called the "ionophone". In the ionophone the sound is radiated directly from an audio modulated radio frequency corona at the apex of an air filled horn. Since there are no moving mechanical parts, except air molecules and ions, the ionophone appeared useful for noise studies where high frequency, high intensity sound waves were needed.

As was mentioned earlier, a high power public address system such as the University B-24 model has certain limitations. One of the most important drawbacks is that such a system has a maximum in its acoustic energy spectrum in a frequency range of 500-5000 cps, whereas small animals may have auditory responses with upper limits close to 100 kc. Thus for animal studies using intense high frequency noise it was necessary to design and construct a high power corona speaker system.

Purpose:

Although commercial low power models were available, it was not known whether the ionophone was purely a heat maintained sound source or was a speaker whose action depended on the direct acceleration of ions. The factors limiting its performance were also unknown. If the ionophone was to prove useful for the production of high intensity acoustic field it was necessary to know the maximum acoustic pressures which could be attained and what the frequency response would be.

Results:

Since detailed results of this work have been submitted, as a special interim report entitled "Corona Type Loudspeaker" by Fujio Oda (1957), only the major accomplishments will be listed. These studies involved the clarification of the mechanism of operation of the corona loudspeaker. Second, the factors which govern its efficiency were determined and critically evaluated. It was also determined what factors were involved in limiting the frequency range. Finally on the basis of the above work a high power, high frequency corona speaker was designed and constructed for use in animal experimentation.

The highest white noise pressure obtained with a 1.3 kw model of the corona speaker was 132 db in a reverberant chamber which had 3×10^4 cubic inches volume. Peak pressures obtained at chamber resonances were 147 db in the middle frequency range.

V. DISCUSSION

The work included in the present report deals mainly with the effects of intense noise on bodily functions other than hearing. It is known that acoustic noise, aside from causing ear damage, can produce a variety of general effects. Noise can cause pain and give rise to subjective symptoms of muscular weakness, nervous irritability and feelings of anxiety. Noise also has an annoyance value and can interfere with speech and communication. Finally, noise elicits physiological changes within animals, including man, as evidenced by the fact that animals respond to noise stimulation. The subject matter of this report deals with the last of these effects, the major aim being to determine whether intense acoustic noise acts as a physiological stress.

The general approaches used in this work involved physiological, biochemical and behavioral effects of intense noise in vertebrate laboratory animals. Specific problems undertaken included the following: 1) flame spectrophotometric analyses of serum electrolytes, 2) eosinophil changes, 3) changes in serum ascorbic acid, plasma cholesterol, and blood sugar, 4) histologic and histochemical changes in adrenals, thymicolymphatic organs, and reproductive organs and, 5) behavior changes associated with noise exposure.

The major findings of these investigations can be summarized in four general statements:

1. Intense noise stimulation at relatively low frequencies (140 db, 150-4800 cps) results in increased adreno-cortical activity. It also causes the appearance of atypical manifestations of "anxiety-like" behavior in rats, mice and guinea pigs.

2. Excessive adreno-cortical activation, however, does not occur with high intensity, low frequency noise stimulation provided the animals are not subjected to extraneous stressful circumstances during noise exposure.

3. Intense acoustic noise acts as a physiological stress to which rats, mice and guinea pigs can satisfactorily adapt. However, although noise cannot be considered harmful (apart from causing ear damage), noise can sometimes contribute to an already existent stressful situation to bring about characteristic manifestations of systemic injury or "shock".

4. Intense noise appears to be more stressful when high frequencies are used (132 db, 2-40 kc) as evidenced by an increased incidence of audiogenic seizures in mouse strains normally considered seizure-resistant.

In discussing the evidence upon which the above conclusions are based, it is desirable to review our own results in light of data collected by other investigators.

Let us first examine the problem of whether noise constitutes a physiological stress. Stress is defined, operationally, by the adaptive response of an organism to some stimulus which has the effect of alleviating the stress-producing conditions. To prove then that noise acts as a physiological stress one needs only to demonstrate that the intricate automatic endocrine mechanisms which constitute the defense resevoir of an animal are being mobilized. In the present studies it was found that acoustic noise stimulation calls forth increased adreno-cortical activity. The main evidence for this was an hypertrophy of the adrenal cortex and a moderate eosinopenia followed by a transitory increase in eosinophils several hours after noise stimulation. It is significant, however, that the decrease in eosinophils was never very great, nor was it a constant feature even following intense acoustic stimulation using low frequency or high frequency noise. On the other hand, the transitory increase in eosinophils as well as increased sudanophilia of the adrenal cortex always occurred following exposure of animals to noise irrespective of what noise pressure level or frequency band was used.

On the basis of the response of the adrenal and also the eosinophil changes it was concluded that both moderately intense acoustic stimulation (110 db, 10-20 kc) and high intensity noise (132-139 db) at high and low frequencies represented a physiological stress to organisms. Our negative findings with respect to tissue pathology, changes in blood ions, blood sugar, serum ascorbic acid and plasma and adrenal cholesterol force us to conclude that the tolerance limits of the animal are not exceeded with intense noise stimulation.

Factual data collected by other workers generally favor the contention that noise is a physiological stress but there is some disagreement on whether noise is harmful to animals including man. Let us first review the evidence which supports some of our own findings.

H. B. Hale (1952) reported increased adreno-cortical activity in workmen working at an aircraft engine testing stand as compared to others engaged in less-noisy engine assembly line work. He concluded "the (eosinophil) pattern obtained from workmen exposed to and accustomed to intense noise is suggestive of a mild stress reaction." Using rats exposed to pure tones (120-140 db, 50-6000 cps) he found the adrenal ascorbic acid response was also indicative of a mild stress reaction.

E. B. Hale (1953) using white noise (121 db, 0-10 kc) also found evidences of increased adreno-cortical activity on male rats (but not in females) as measured by increases in the weight of the adrenal glands and in the size of the active zones of the adrenal cortex. No evidence was obtained of systemic pathology resulting from excessive adreno-cortical activation.

Bugard (1951, 1953) ran a series of studies using dogs, rabbits and guinea pigs exposed to intense noise (130 db, 25-55 kc). His pertinent findings following noise stimulation can be summarized as follows: 1) slight (but not significant) rise in blood sugar, 2) eosinopenia and lymphopenia. The eosinopenic response was not obtained consistently but it always preceded a transitory increase in eosinophils of considerable magnitude. 3) no change in urinary excretion of 17-ketosteroids, 4) hyperactivity of the thyroid, adrenals and anterior pituitary (acidophilic components) determined histologically. He concluded that except for the fatal lesions produced by Pimonows siren (intensity levels 140-160 db causing production of severe body burns), there were no pathological changes produced by intense acoustic stimulation. He further stated his results indicated that the limits of physiological activity which predominated at the level of the anterior pituitary and adrenal cortex were not exceeded.

Fortier and his coworkers (1951) have also reported increased adreno-cortical activity associated with sound exposure but made no attempt to examine whether the sound was acting as a severe or harmful stress.

Some of the above observations, including our own results, are contradicted by the findings of Miline (1951, 1954) who has described the various types of pathological changes in adrenals, thyroid and heart following exposure of animals to noise alone or noise associated with vibrations. In an earlier report Miline and Kochak (1951) described the adrenal response of animals as follows: hypertrophy and degranulation of cellular elements in both the adrenal cortex and adrenal medulla. In a second report (Miline, 1952) he reports regressive changes in thyroids. In more recent reports he has described pathological changes in cardiac musculature, and also in reproductive organs. All of his data are presumably collected from the same group of animals exposed for about two weeks to the noise and vibrations of a railroad boiler factory ("ateliers bruyant de chaudronnerie de chemin de fer"). Although he states that noise with physical vibrations is more stressful than noise alone he maintains that noise by itself is a severe stress stimulus similar to other types of stress stimuli described by Selye. For example, among the pathological effects attributed to noise alone include reduction in spermatogenesis, and oligospermia. In none of the reports is the exact nature of the noise situation specified.

It stands to reason from the foregoing discussion that to assert that noise is harmful one has to establish that the animal's capacity to withstand noise is exceeded. The data accumulated from our studies would indicate that this is not the case. On the contrary, all of the evidence indicates that the mobilization of defense endocrine mechanisms enables the organism to successfully adapt to intense acoustic stimulation. Sometimes physiological researchers lose sight of the primary function of the general adaptation syndrome. By merely demonstrating the existence of a stress, it is assumed that under unrelieved stress the animal's defense mechanisms will ultimately break down. Fortunately, this is obviously not true as evidenced by the ability of animals, including man, to survive the variety of stresses that represent an integral part of the daily environment.

One should proceed with caution before drawing a generalization that noise is never harmful to bodily functions other than hearing. In the laboratory, we attempt to study noise as a single component divorced from other environmental stresses which could bias our results. This situation would seldom hold under natural conditions, for wherever one encounters intense sound pressure levels there are often many other types of stressful stimuli, whether it be in a boiler factory such as that referred by Miline or around turbo-jet testing cells where exhaust fumes and physical exertion are stress companions of noise. Evidence that noise can aggravate an already existent stress was obtained by Hale (1953) who found a reduced survival time of fasting rats subjected to noise. This was confirmed in the present report where it was found that noise exposure aggravated the stress effect of food restriction in mice. Miline's claim regarding the damaging role of noise on animals are thus subject to considerable question; not only are the stimulus conditions not specified, but no attempt was made to control other variables that may have accounted for the effects observed.

This brings us to the problem of noise and behavior. Much has been written on how noise affects human behavior and an excellent summary of the effects of noise on work performance, noise as a source of annoyance and general problems of methodology can be found in a report by Rosenblith (1953).

Among the subjective symptoms listed by Parrack (1948, 1949) for humans are the following: (a) weakness in the knees or apparent general weakening of body supporting musculature, (b) nausea and vomiting, (c) severe headaches, (d) hyper-irritability, (e) extreme fatigue. Others have also reported transitory symptons of dizziness, unsteadiness and mental aberration in ground crew working near turbo-jet engines. European authors have also described these symptoms in what is referred to as "le syndrome traumato-sonore"

(Bugard, 1951, 1953). It is apparent that such symptoms, however real, are nonetheless difficult to evaluate as is the noise attribute of annoyance.

In our studies of the behavioral effects of intense acoustic stimulation in laboratory animals an attempt was made to quantify certain activities which appeared atypical either in character or frequency of occurrence. The behavioral responses to noise which represented striking deviations from normal behavior were a reduction in general activity and an increase in what was arbitrarily referred to as excitability or a state of anxiety. The reduction in general activity occurred as a consequence of a freezing response of individuals or huddling of several individuals into a closely knit group. The adaptive value of this latter behavior is apparent since not only does it serve to help insulate animals from noise stimulation, but may also conserve energy normally expended in movements about the cage.

In contradistinction to this "withdrawal" or escape response from noise stimulation, there occurred a marked increase in washing and grooming activities during noise stimulation especially in mice and rats. One reason for the increased face washing and genital washing can be explained as a response to sensory skin vibrations. Davis (1949) reports that sound levels of about 120 db stimulate receptors in the mouth, nasal passages and external ear canal in humans. It is quite likely that the surfaces likely to be irritated the most by intense noise in rodents would be the mucous epithelium of the nasal, oral and genitourinary orifices. The increased incidence of grooming of the dorsal and abdominal skin could also be a logical consequence of vibratory stimulation of body receptors. In humans the tactile sensory threshold for sound is about 130-140 db. Viewed in this light, the behavioral response of the animals to acoustic noise appears to be adaptive rather than symptomatic of a neuropathic state due to excessive sensory stimulation.

In brief, the endocrine response, changes in blood components and behavioral expressions following intense noise stimulation all indicate that animals successfully adapt to intense acoustic stimulation. However, the fact that noise elicits a defense response would make it reasonable that there are levels of acoustic noise that will overtax the homeostatic adaptive mechanisms. Considerable work will be necessary to define the tolerance limits of animals to noise both alone and in situations where noise is only one of several stressful stimuli.

VI. SUMMARY

The effects of intense acoustic noise on bodily functions, other than hearing, were investigated using rats, mice and guinea pigs. Two levels of noise stimuli were employed, moderately intense noise of about 110 db re 0.0002 dynes/cm^2 and intense noise with pressure levels of about 132-140 db. The frequency ranges employed were 10-20 kc, 150-4800 cps and 2-40 kc. Responses of animals to single noise burst were studied as well as the effects of chronic noise exposures under various conditions.

Intense acoustic noise was found to act as a physiological stress in laboratory animals as evidenced by an increase in adreno-cortical activity associated with changes in circulating blood eosinophils. No evidence was obtained, however, which indicated overactivation of the pituitary-adrenal axis. Data to support these conclusions came from histologic and histochemical analyses of adrenals and target organs influenced by adrenal secretions, from quantitative studies of blood eosinophils, serum electrolytes, plasma ascorbic acid and cholesterol and also from the adaptive behavioral response of animals in intense noise situations.

Although noise by itself was not harmful, it was shown that intense noise can reduce the survival time of animals subjected to the stress of food restriction. It was also shown that intense noise, especially at higher frequencies, will increase the incidence of audiogenic seizures in mouse strains which are normally seizure-resistant.

It was concluded that the attendant adrenal and eosinophil changes, and behavioral responses reflect successful adaptation to noise rather than an overtaxing of the animal's defense mechanisms.

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VIII. APPENDIX

The following set of figures is included for those who might be interested in various details of the two main experimental set-ups used to produce high intensity noise at low and high frequencies.

Figs. 1A through 6A refer to the high power University B-24 sound equipment and include a block diagram of the sound apparatus, a sketch of the six cage reverberant chamber, results of noise level measurements and octave band noise analyses of the chamber, and background noise level measurements inside and outside the sound insulated room which was built to house the speaker and test chamber.

The rest of the figures pertain to various items of equipment designed and constructed for the corona loudspeaker system. These include a block diagram of a 60 watt model speaker system, circuit diagrams of the oscillators and modulators used with 60 watt and 1.3 kilowatt speaker models, various types of speaker models tested and frequency response characteristics of some of these models.

The corona loudspeaker which was employed for studies of intense high frequency noise was the 1.3 kw, 28 Mc model speaker. Fig. 18A shows the frequency response characteristics of this speaker; Figs. 19A and 20A show plots of acoustic pressure as a function of the r. f. power and air flow (used in cooling of the electrode). The design of the speaker and the electrode used are shown in Figs. 21A and 22A where the units are in millimeters. The speaker itself was made of vycor glass whereas the electrode was made of platinum set into a water and air-cooled casing made of copper and brass.

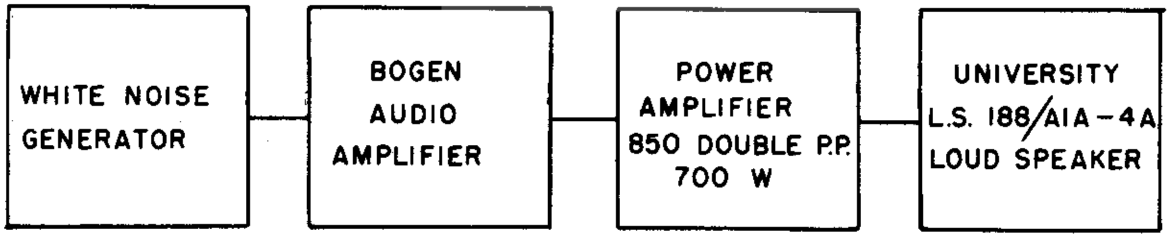


FIG. 1A, APPARATUS USED TO GENERATE NOISE

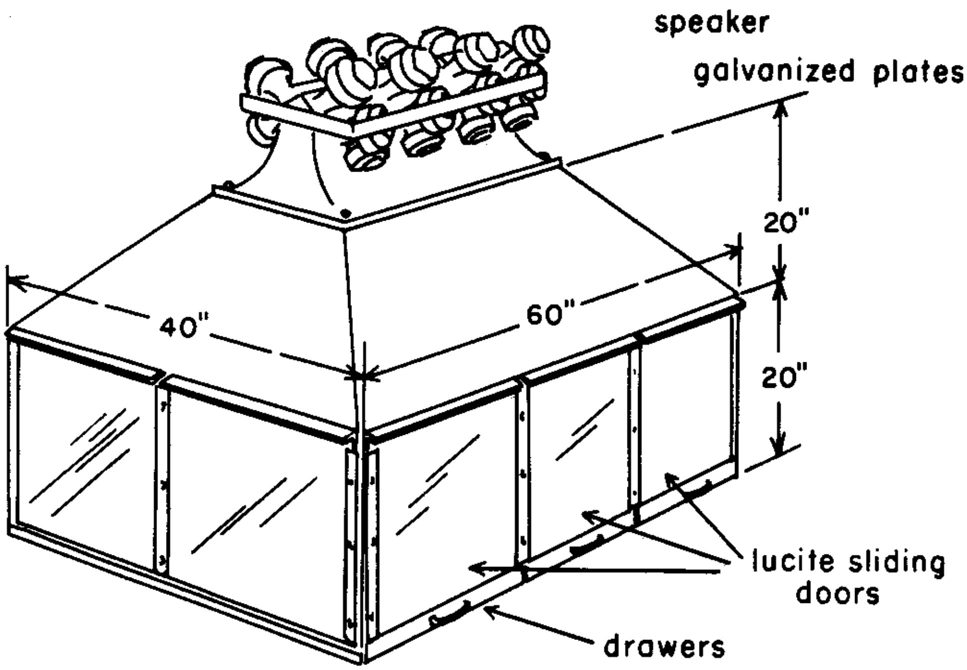


FIG. 2A, SKETCH OF THE REVERBERANT CHAMBER AND THE SPEAKER

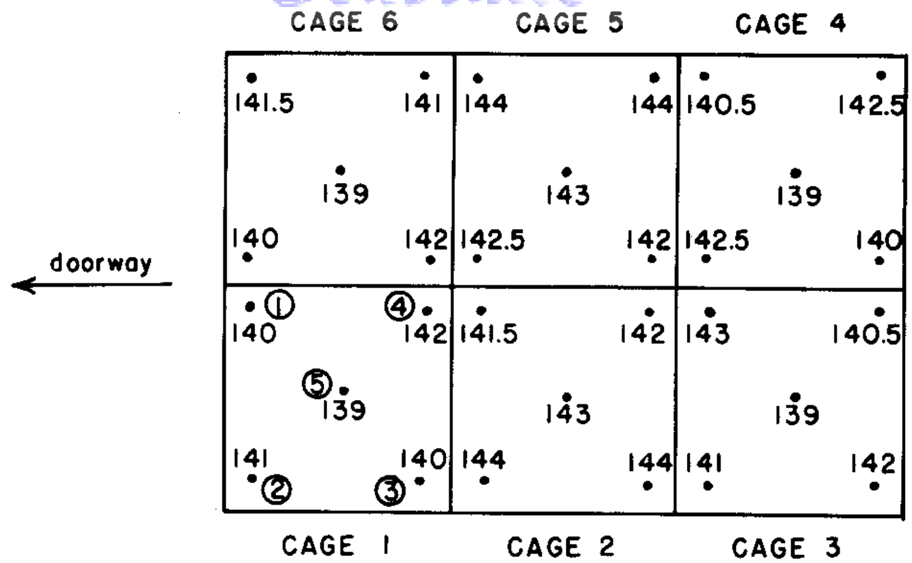


FIG. 3A, THE NOISE LEVEL IN DECIBELS WITHIN INDIVIDUAL CAGES IN THE REVERBERANT CHAMBER (circled numbers correspond to location on the screen floor where measurements were taken).

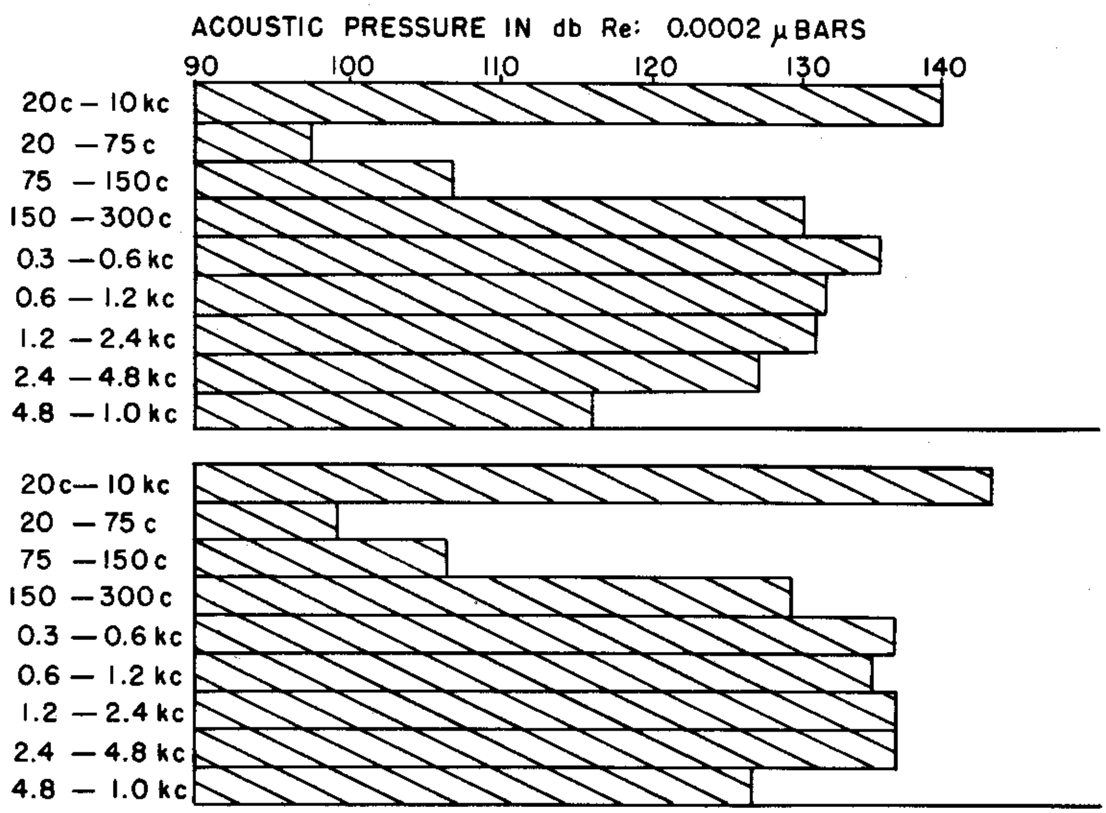


FIG.4A, OCTAVE BAND NOISE ANALYSIS FOR CENTRAL PART (POSITION 5) OF CAGES 1 AND 2.

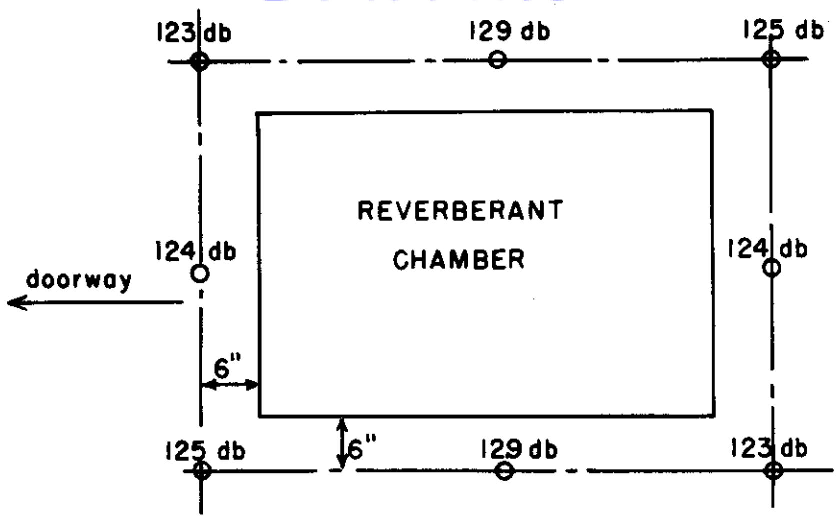


FIG. 5A, NOISE LEVELS OUTSIDE THE REVERBERANT CHAMBER

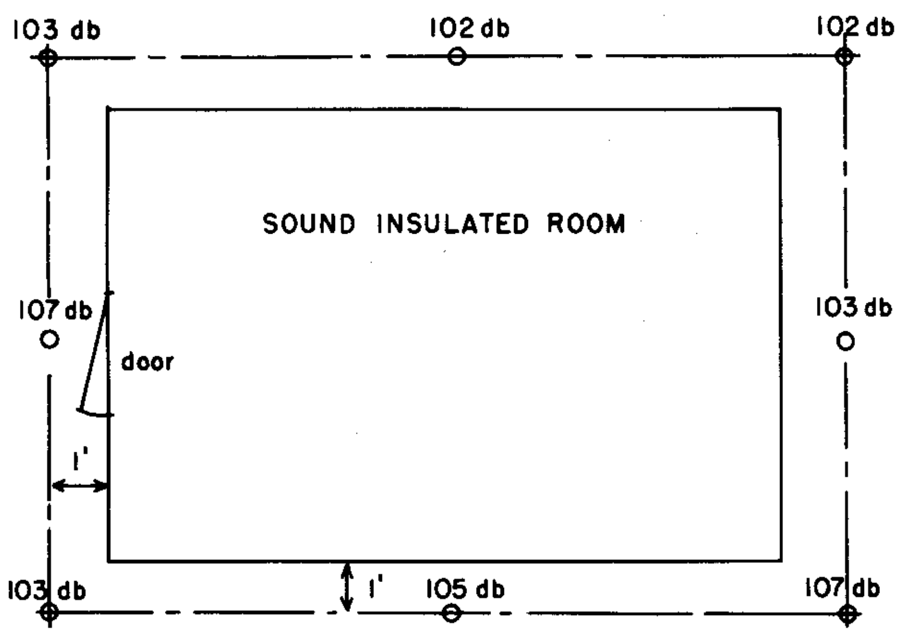


FIG. 6A, NOISE LEVELS OUTSIDE THE SOUND-INSULATED ROOM

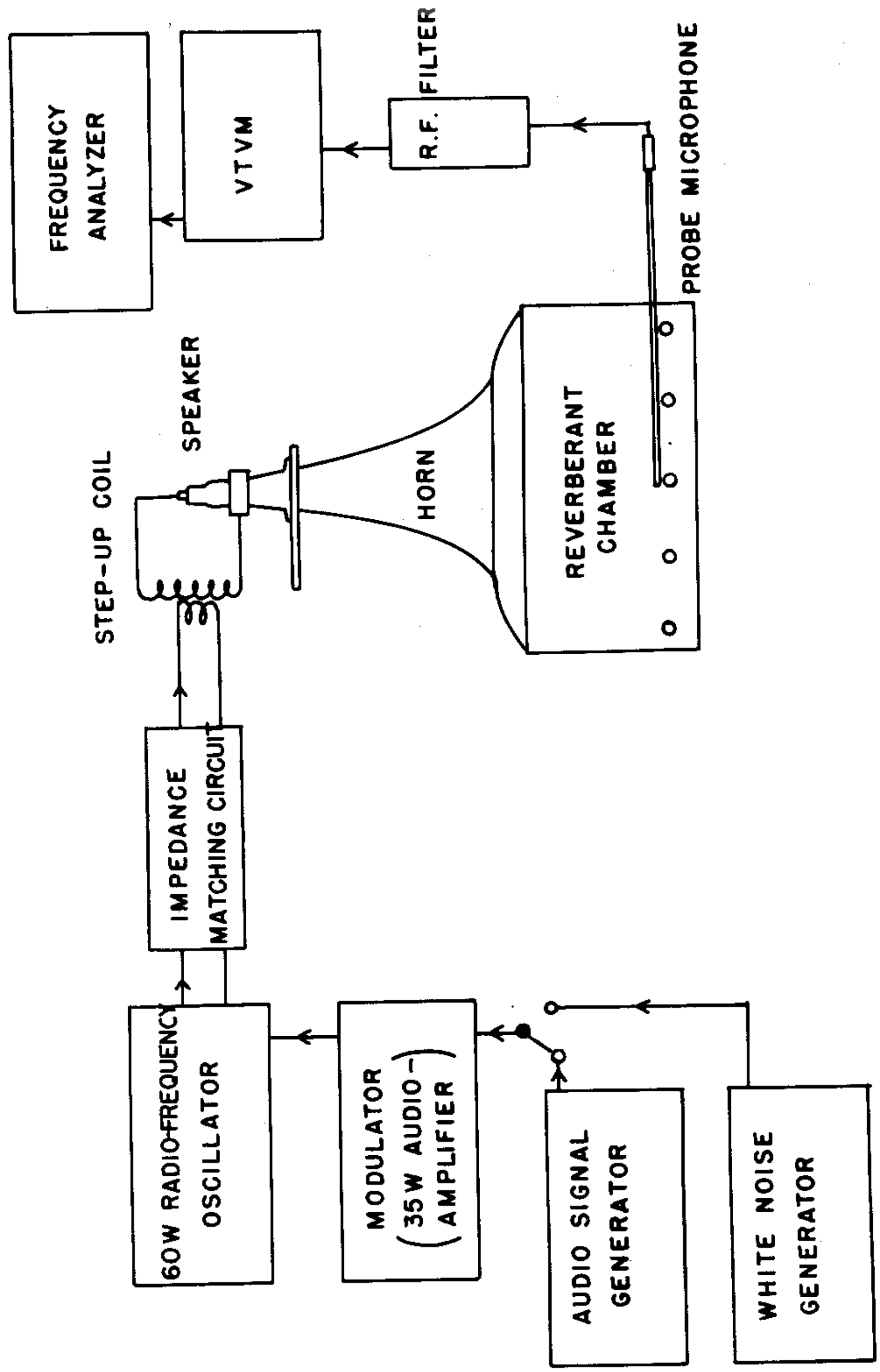


FIGURE 7A, BLOCK DIAGRAM OF 60 W MODEL SPEAKER SYSTEM

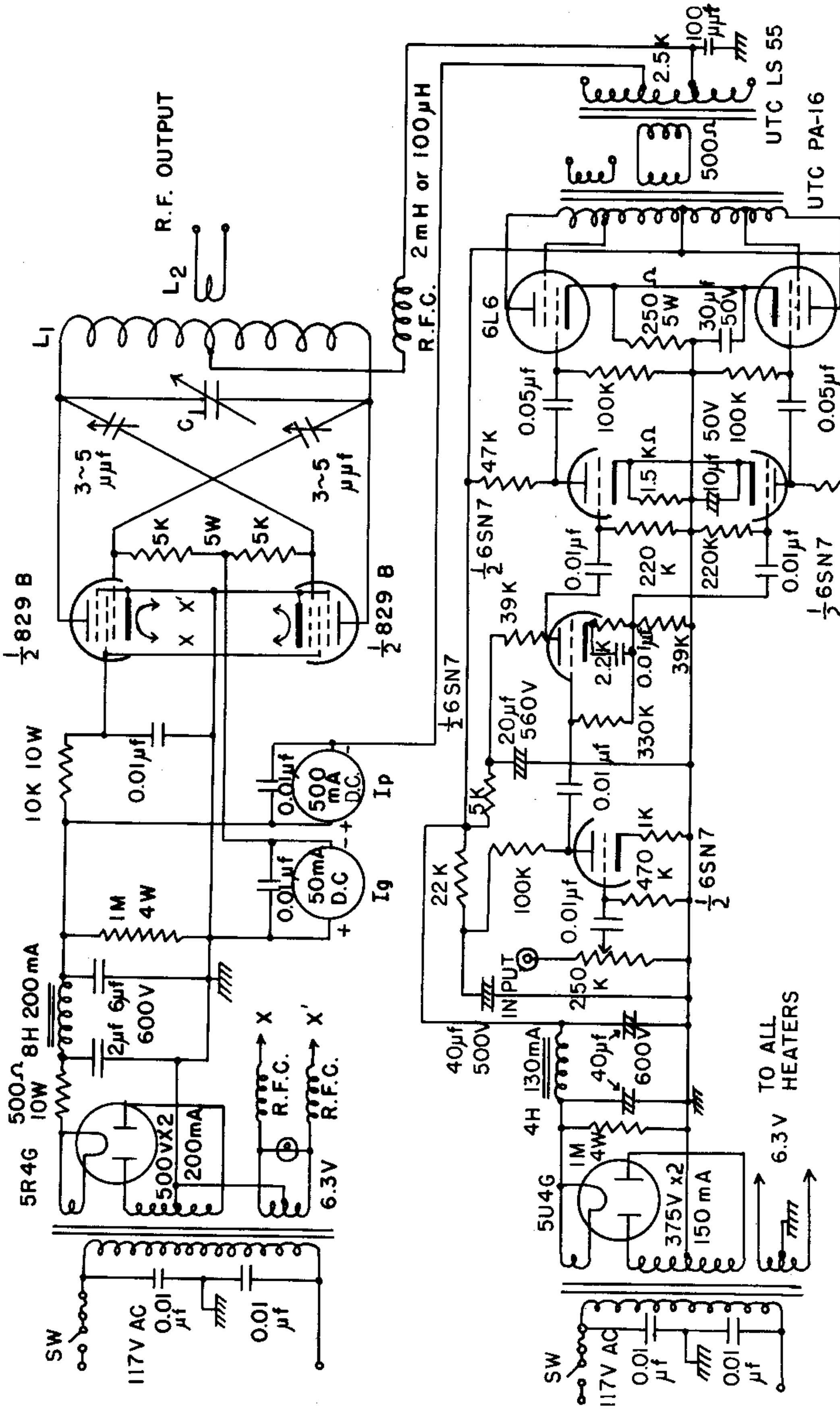


FIGURE 8A, CIRCUIT DIAGRAM OF OSCILLATOR AND MODULATOR FOR 60W MODEL

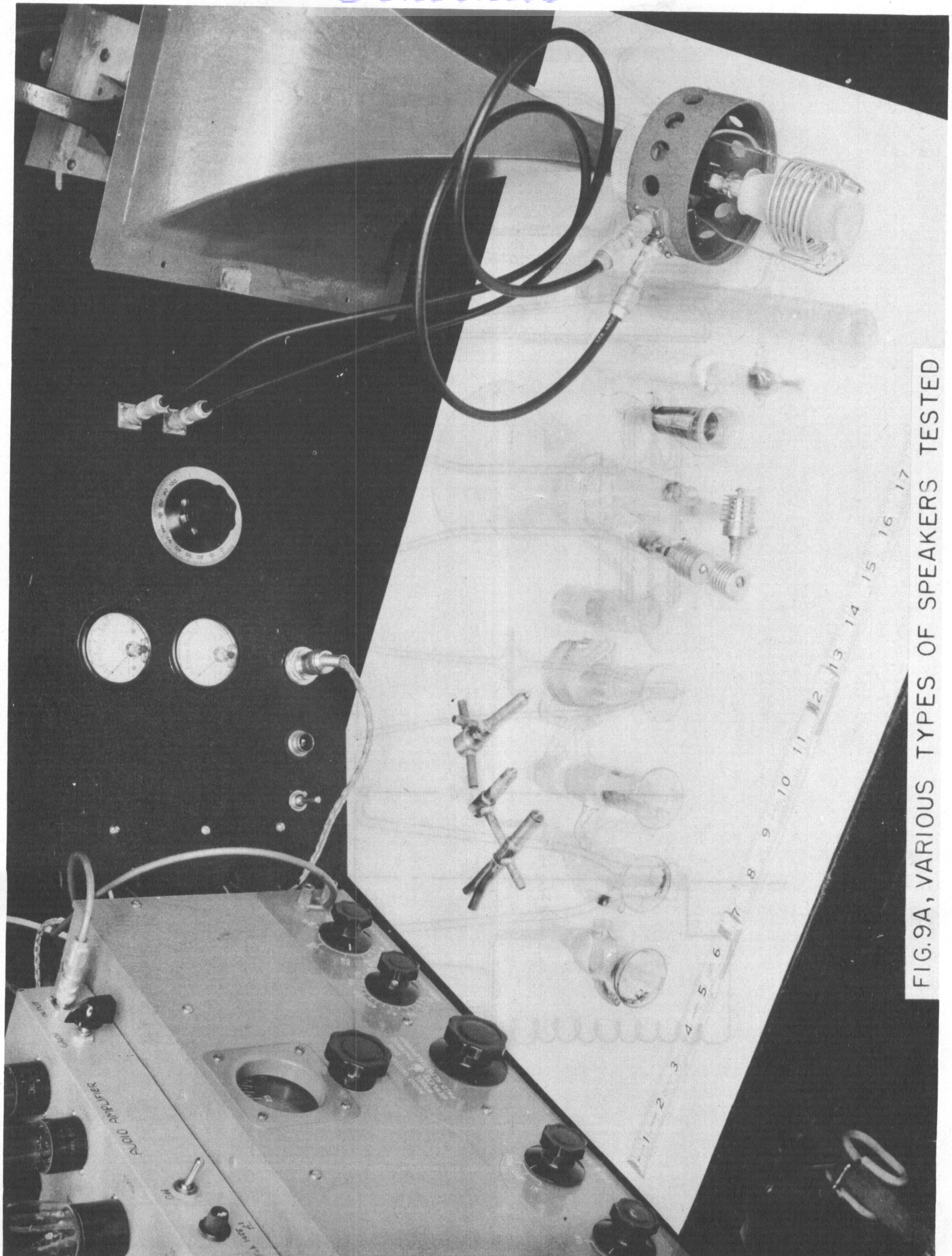


FIG.9A, VARIOUS TYPES OF SPEAKERS TESTED

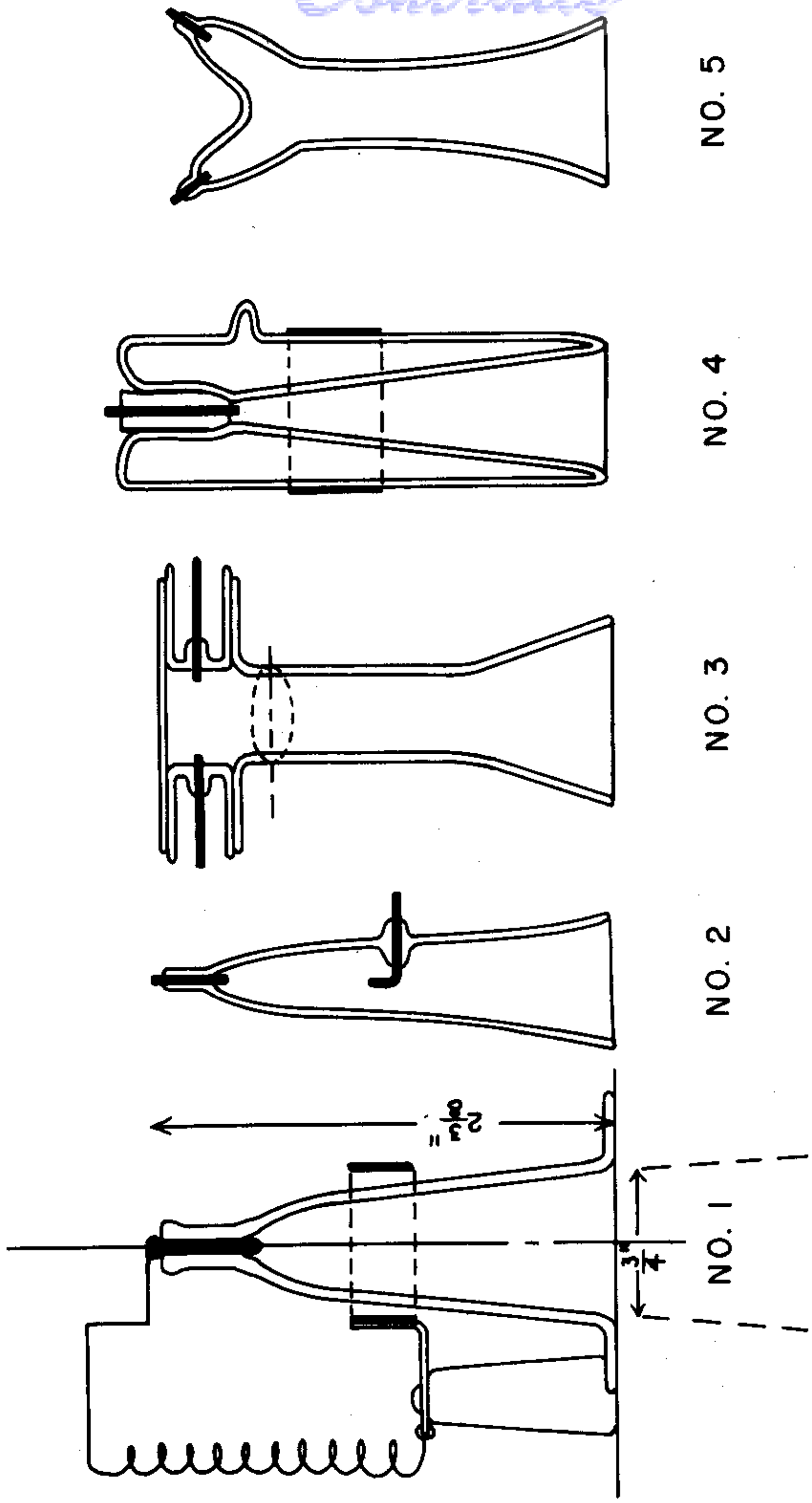


FIGURE 10A, VARIOUS TYPES OF SPEAKERS TESTED FOR 60W MODEL

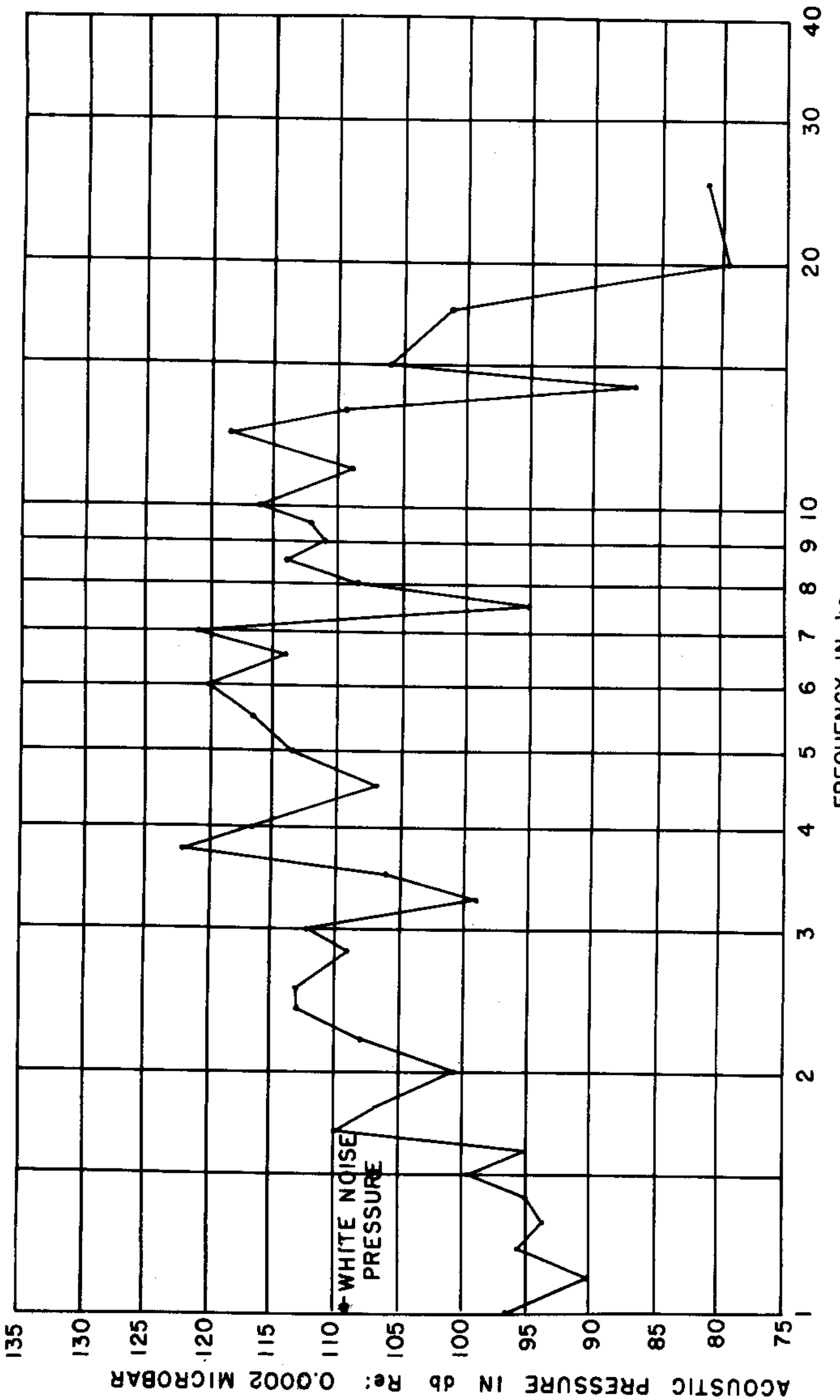


FIGURE 11A, FREQUENCY RESPONSE OF 60 W, 1 Mc MODEL SPEAKER

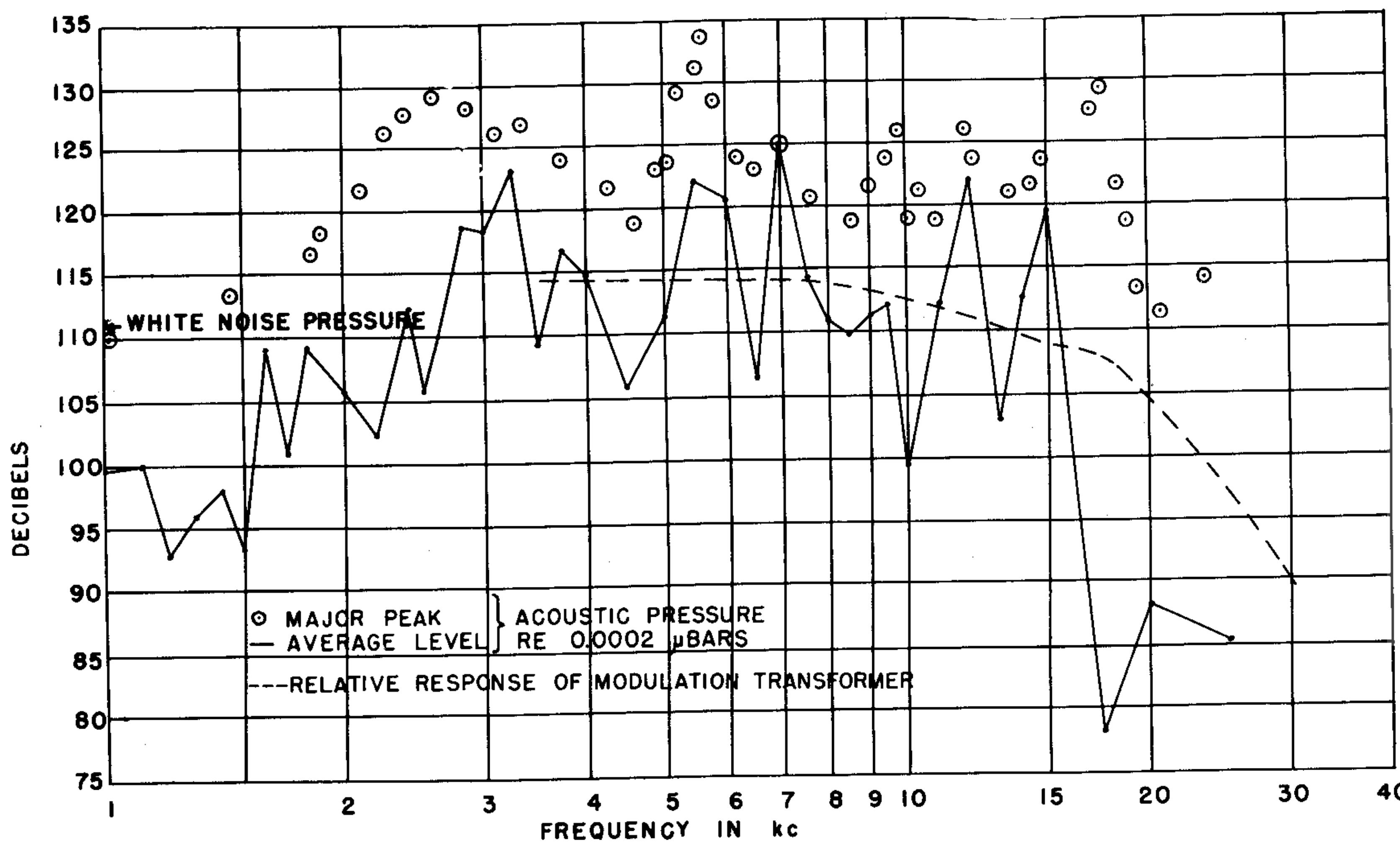


FIGURE 12A, FREQUENCY RESPONSE OF 60 W, 20Mc MODEL SPEAKER

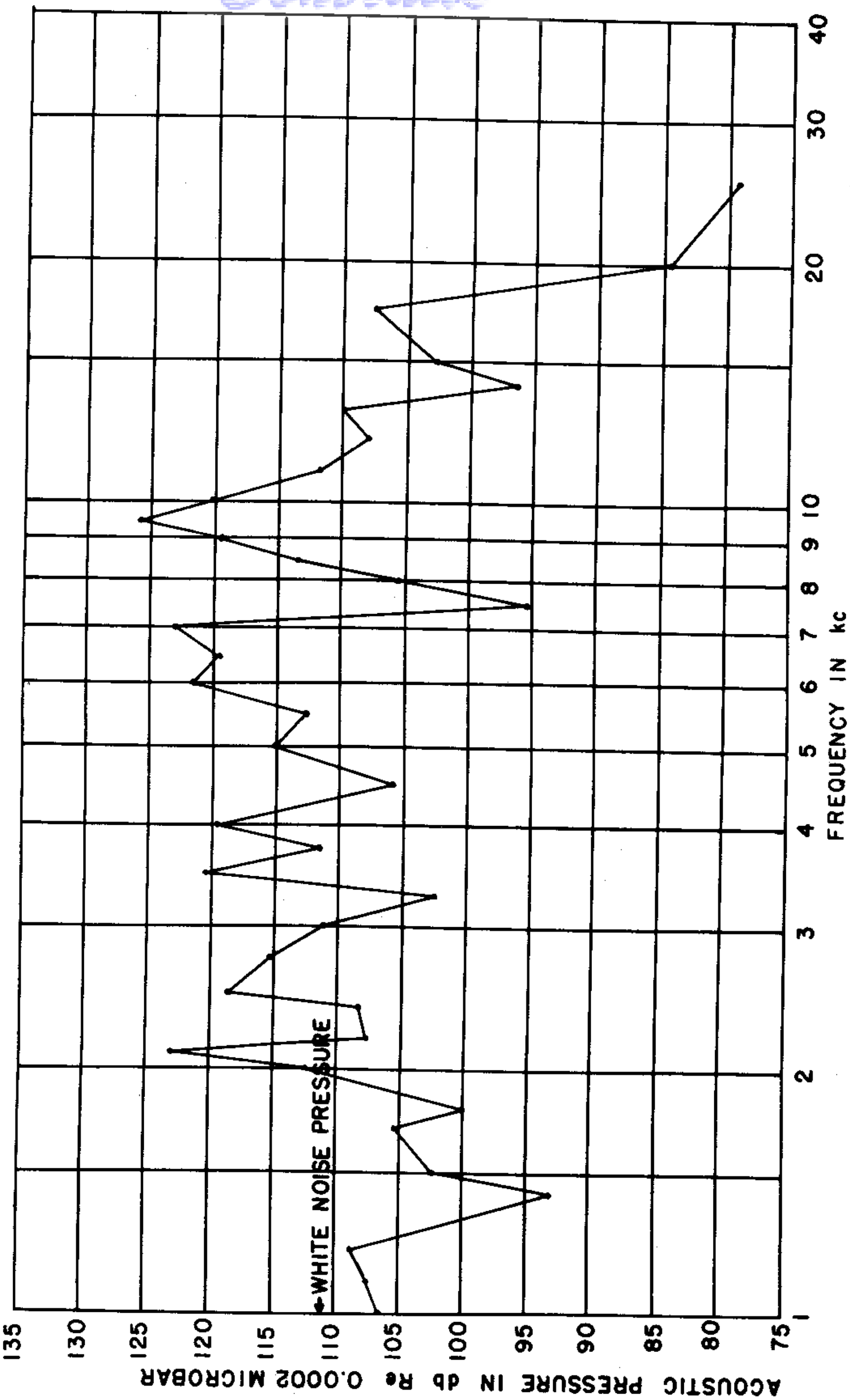
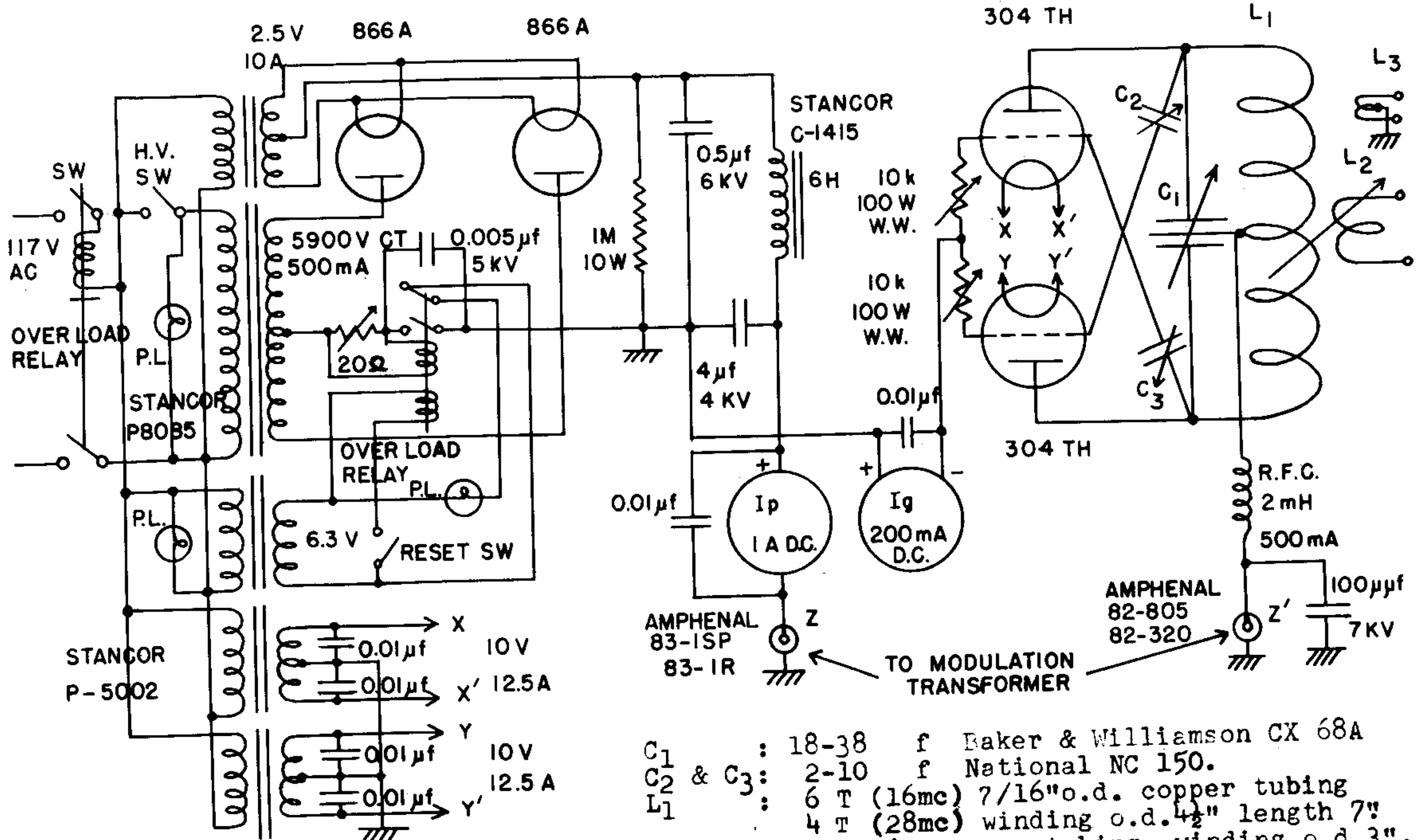


FIGURE 13A, FREQUENCY RESPONSE OF 60 W, 105 Mc MODEL SPEAKER



C_1 : 18-38 f Baker & Williamson CX 68A

C_2 & C_3 : 2-10 f National NC 150.

L_1 : 6 T (16mc) 7/16"o.d. copper tubing

4 T (28mc) winding o.d.4½" length 7"

L_2 : 2 T ¼" copper tubing. winding o.d.3". length 1".

L_3 : Pick-up coil. 5T. 1" o.d. #22 wire.

FIGURE 14A, CIRCUIT DIAGRAM OF OSCILLATOR FOR 1.3 KW MODEL

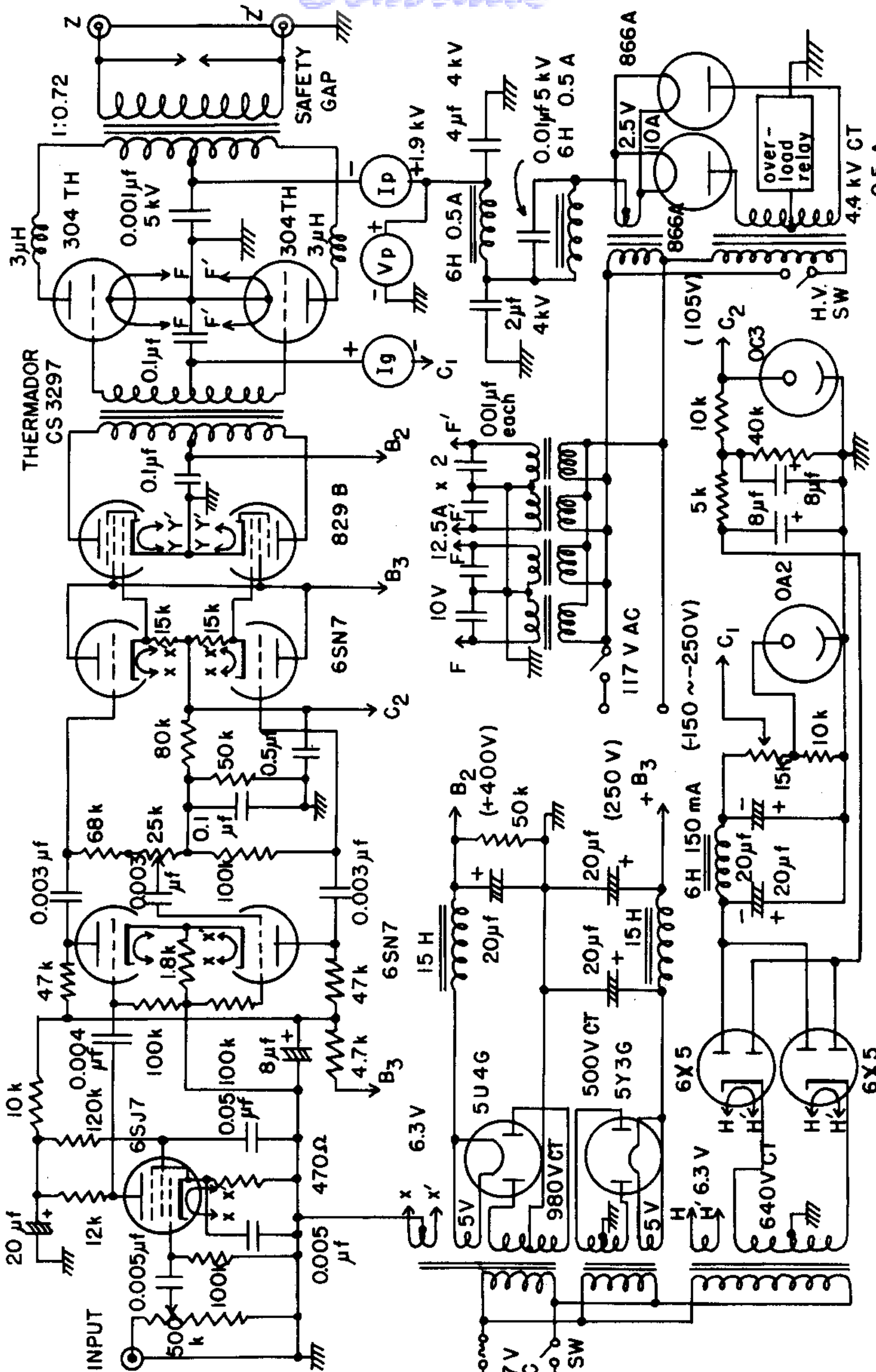


FIGURE 15A, CIRCUIT DIAGRAM OF MODULATOR FOR 1.3 KW MODEL

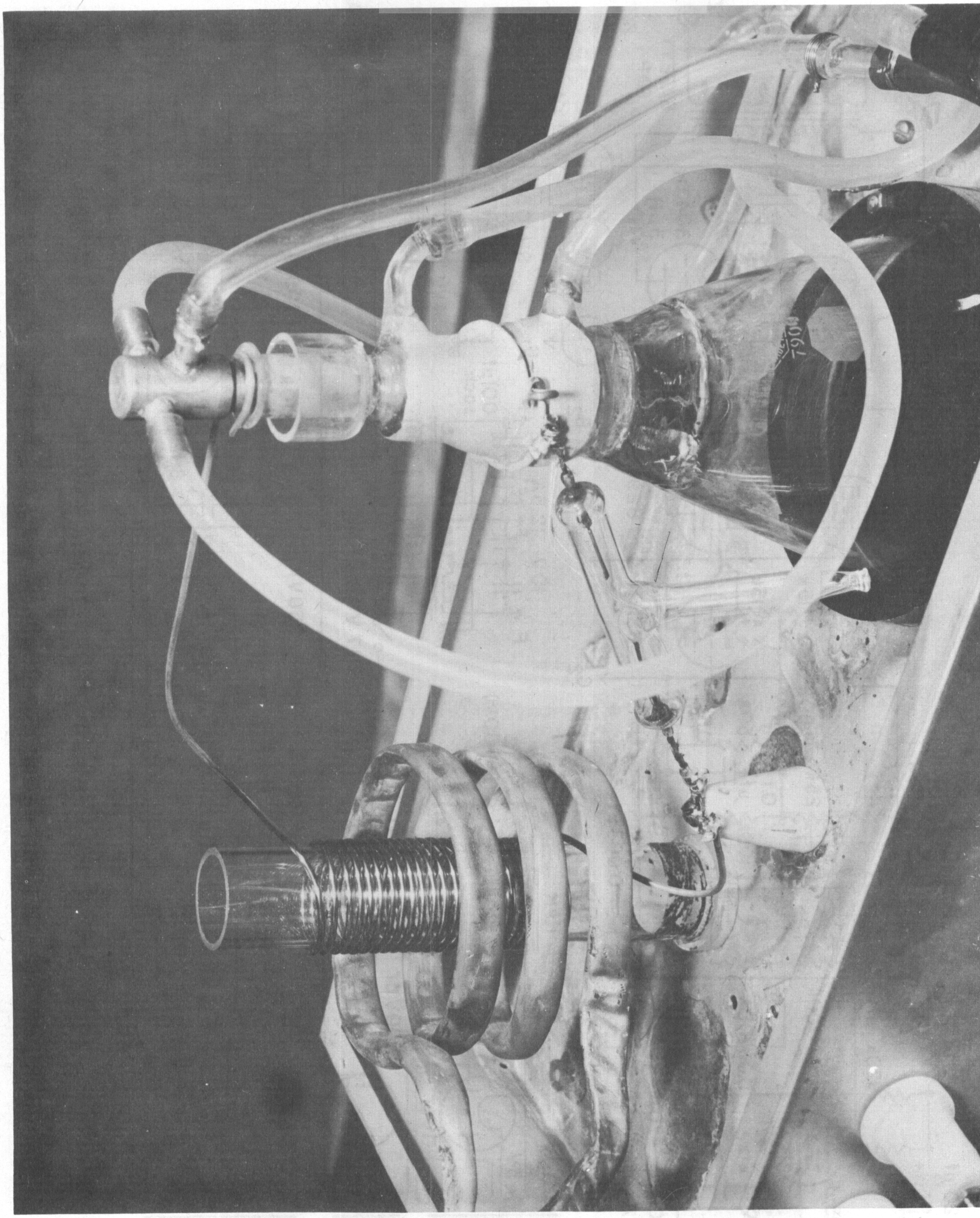


FIG. 16A, SPEAKER AND STEP-UP COIL ASSEMBLY FOR 1.3 KW MODEL

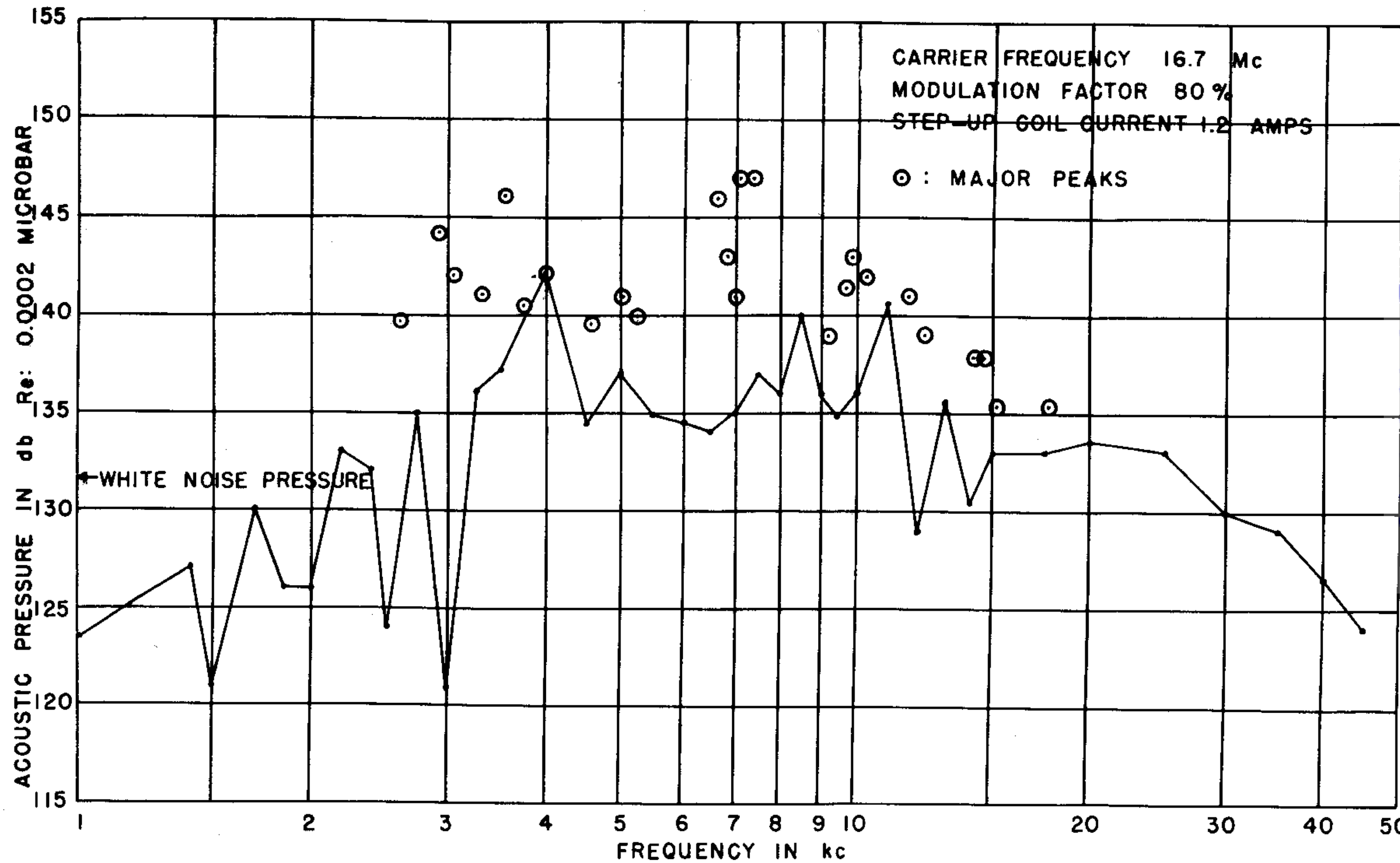


FIGURE 17A, FREQUENCY RESPONSE OF 1.3 KW, 16.7 Mc MODEL SPEAKER

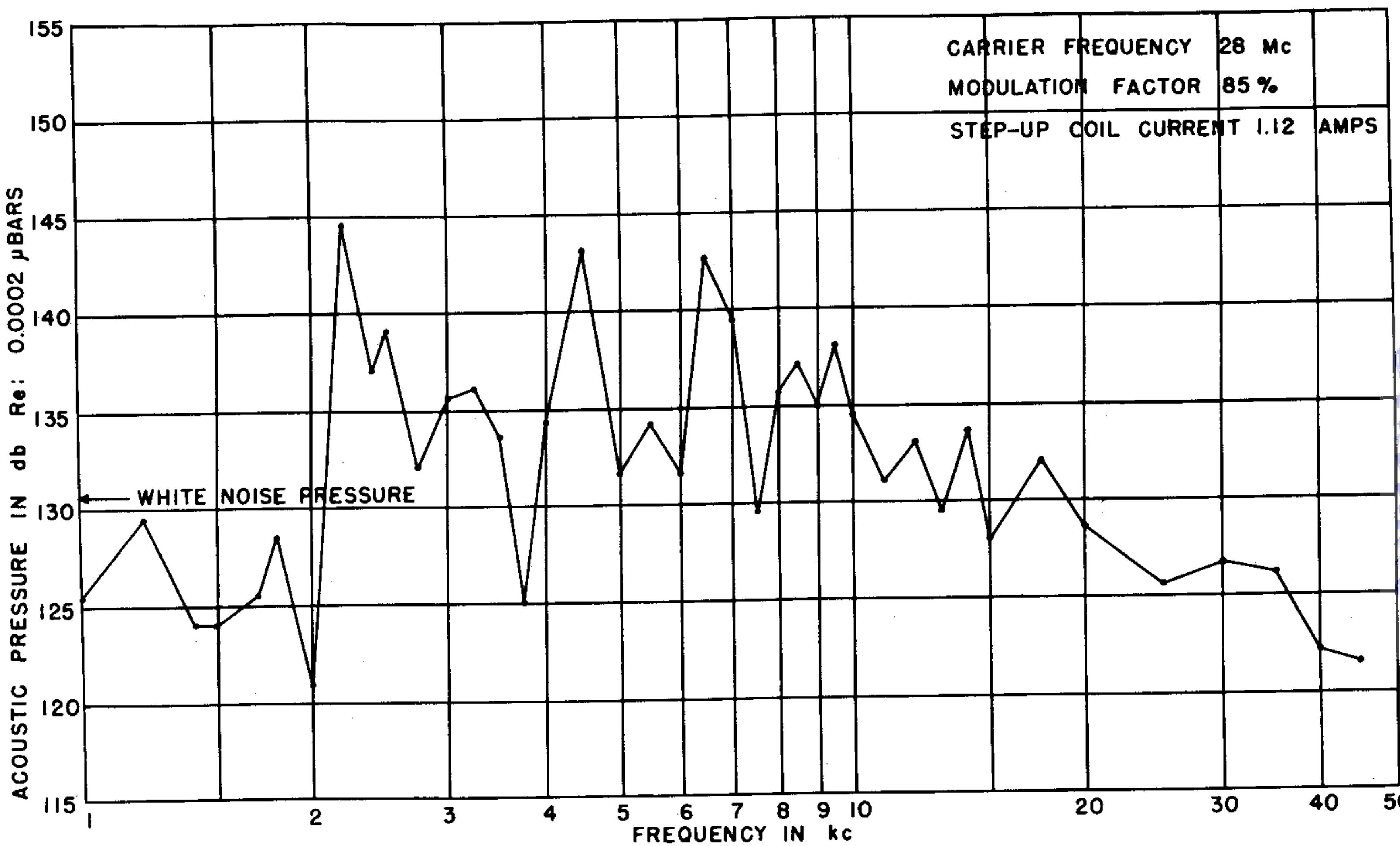


FIGURE 18A, FREQUENCY RESPONSE OF 1.3 KW, 28 Mc MODEL SPEAKER

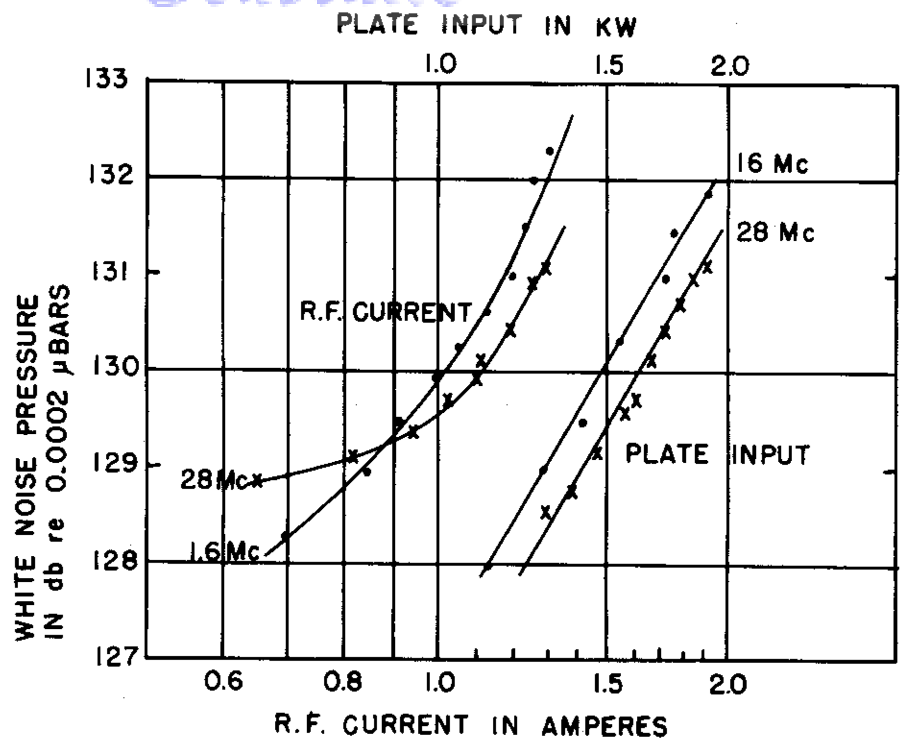


FIGURE 19 A, R.F. POWER VS ACOUSTIC PRESSURE

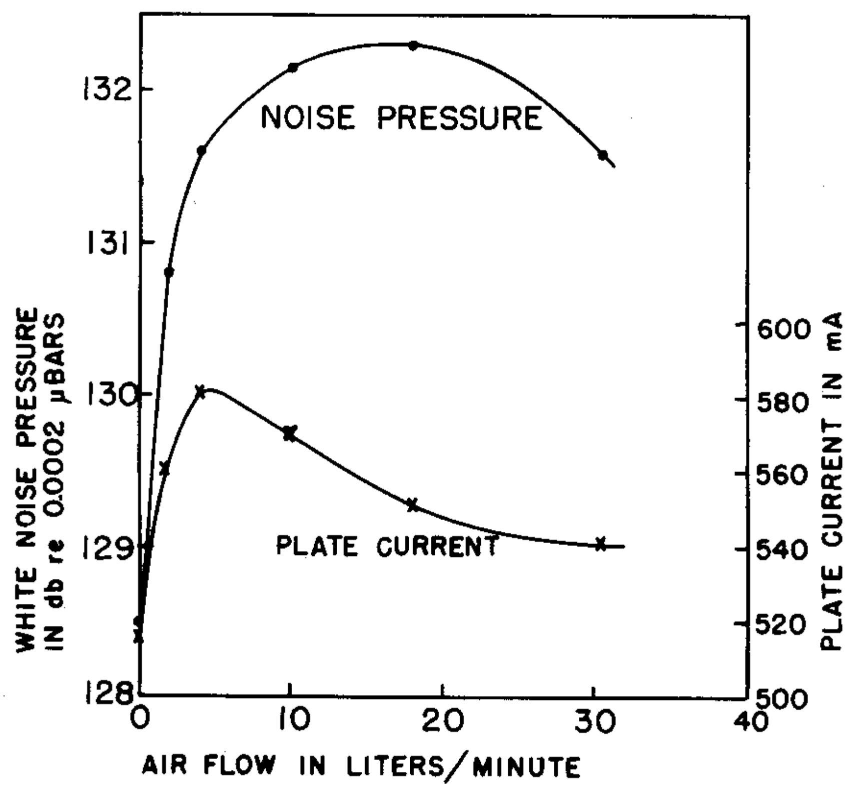


FIGURE 20 A, AIR FLOW VS ACOUSTIC PRESSURE

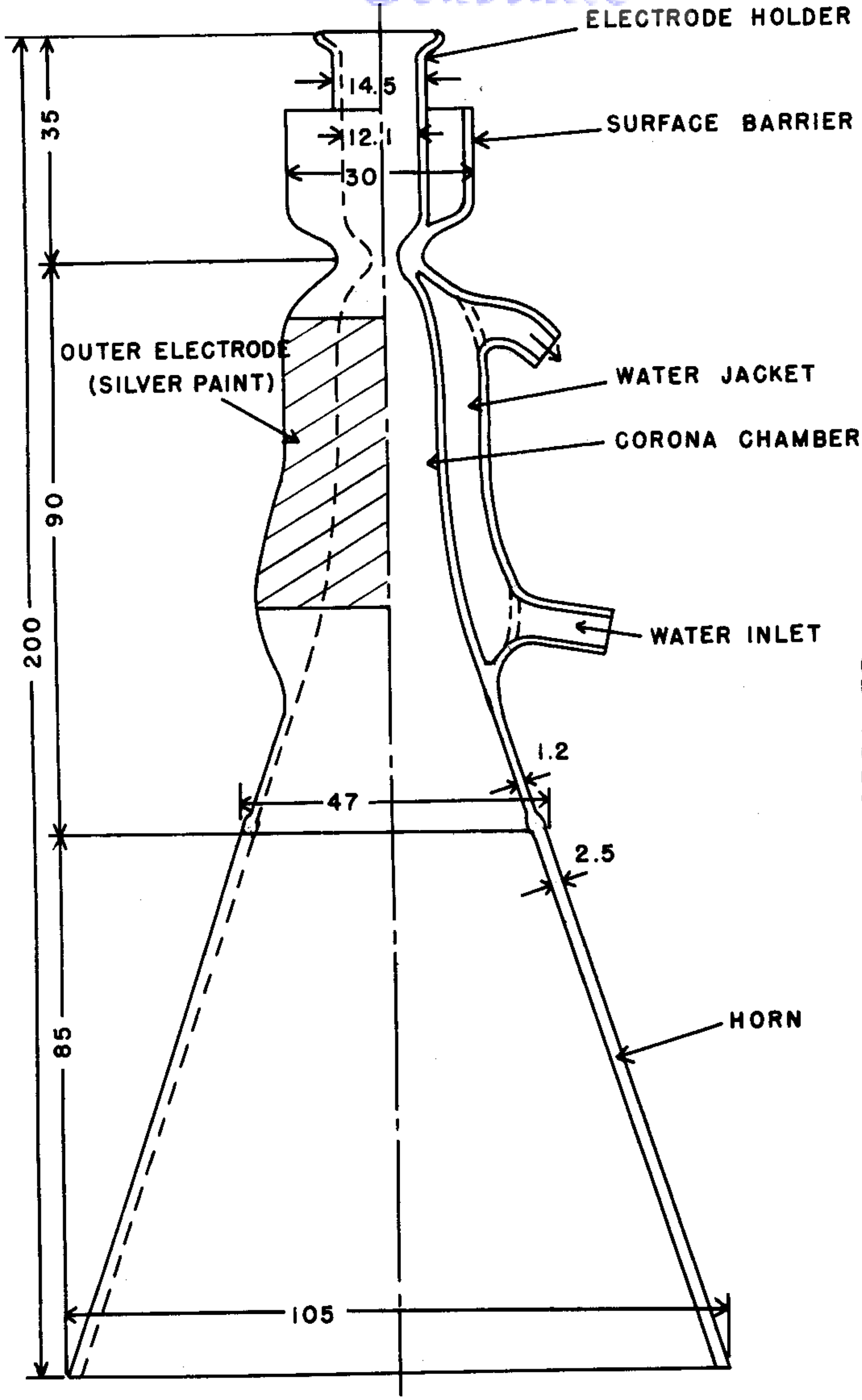


FIGURE 21A, DESIGN OF SPEAKER

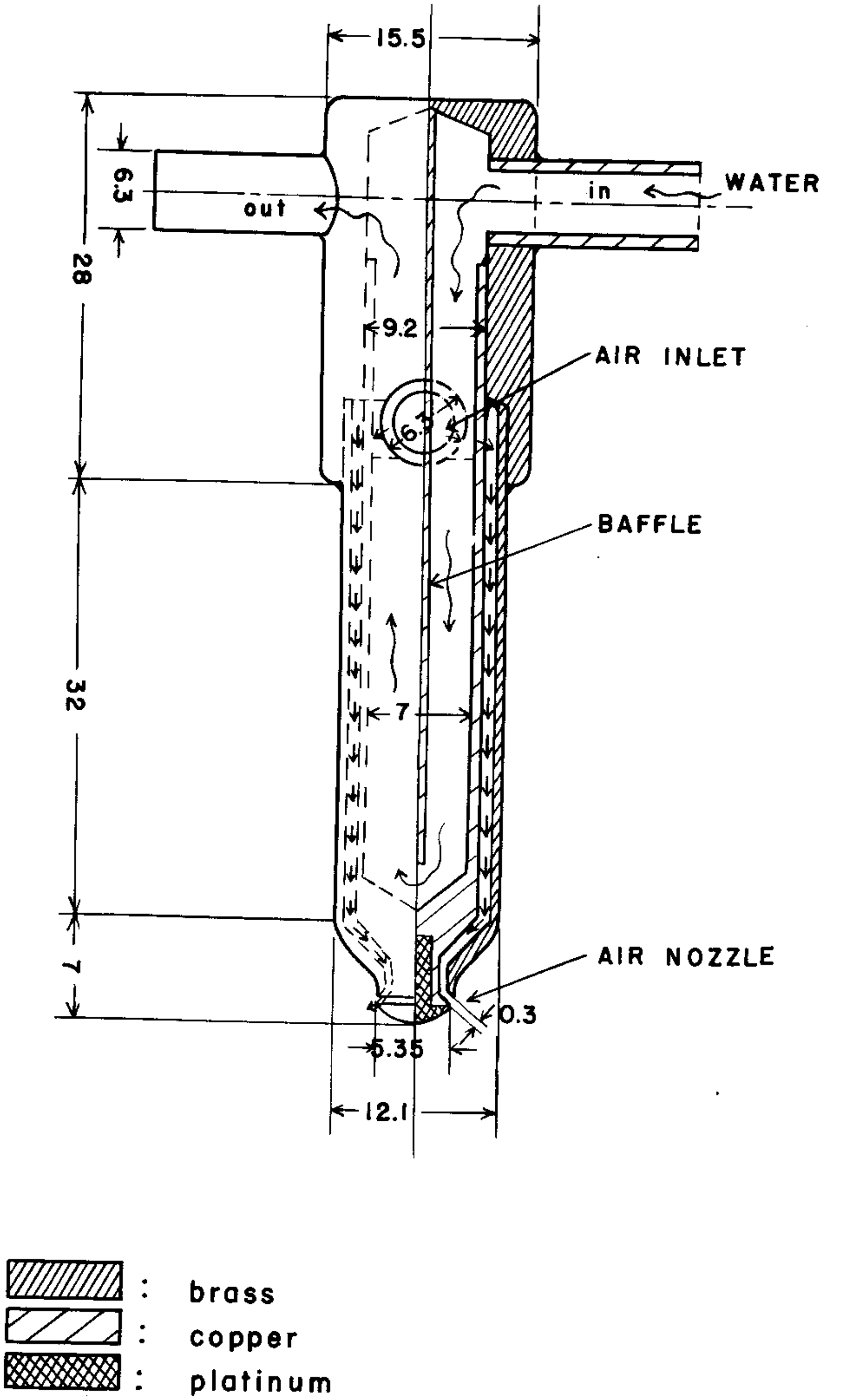


FIGURE 22A, DESIGN OF CENTER ELECTRODE

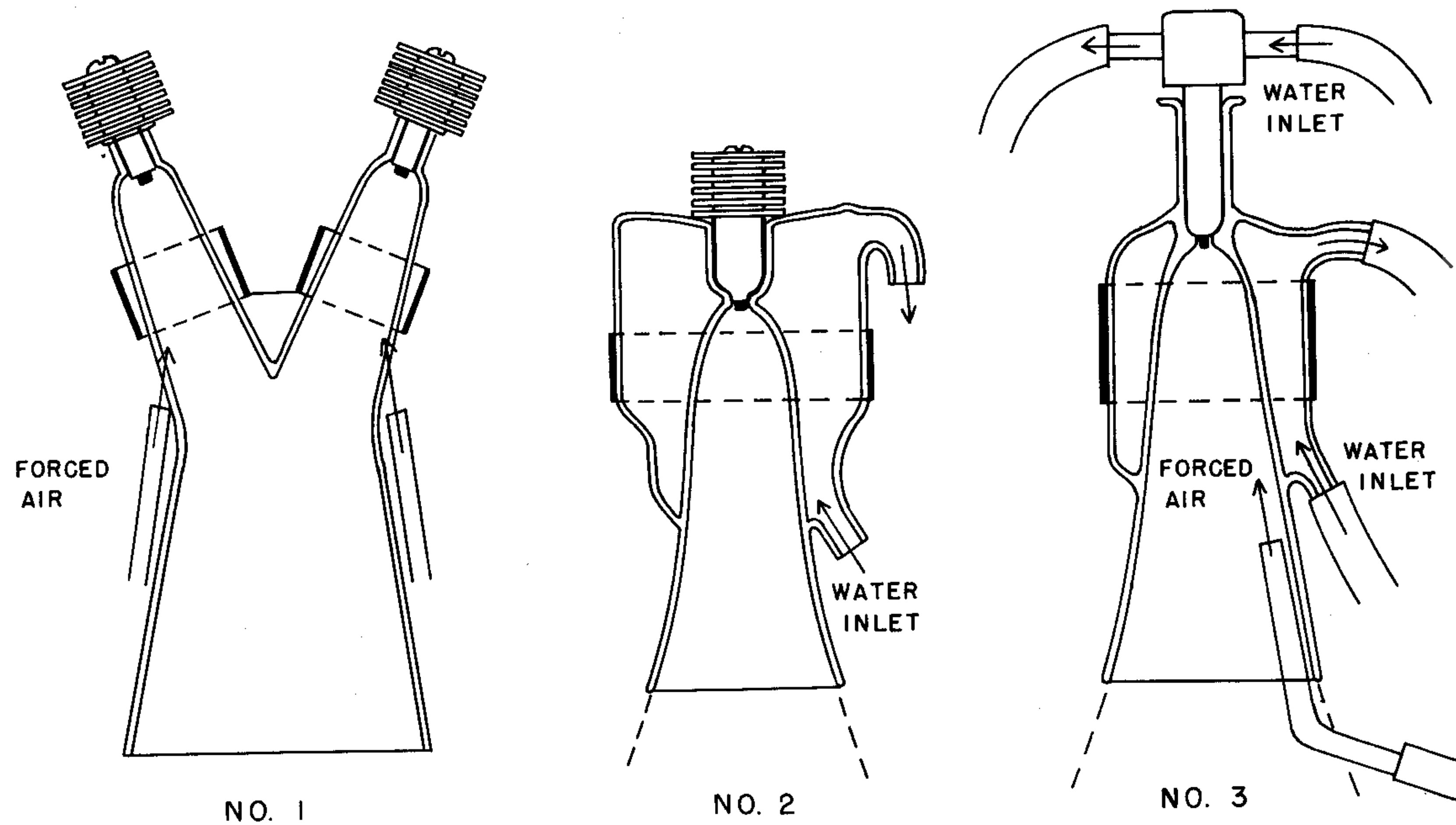


FIGURE 23A, VARIOUS SPEAKERS TESTED FOR 1.3KW MODEL