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Effects of an Acoustic Stimulus upon Growth, Antibody Synthesis and Leukocyte Values

CALVIN R. KOEHLER, DAVID R. GRIFFITH and CHARLES J. ELLIS¹

KOEHLER, CALVIN R., D.R. GRIFFITH, AND C. J. ELLIS (Department of Zoology, Iowa State University, Ames IA 50011). Effects of an Acoustic Stimulus upon Growth, Antibody Synthesis and Leukocyte Values. Proc. Iowa Acad. Science 83(3):102-111, 1976.

Physiologic effects of an acoustic stimulus of 1.0 Khz at 96 db(A) upon female hooded rats are discussed. Six possible reasons are offered for failure to attain statistical significance, except in intermediate aspects, between control and experimental animals.

Noise pollution in our over-polluted world is an unfavorable characteristic of everyday life which is steadily increasing. Dr. Vern Knudsen, acoustician and U.C.L.A. professor, suggested some sound levels are up 20 decibels over those of 20 years ago, representing a 10-fold increase in sound pressure bearing on our eardrums (Lipscomb, 1974). Further increase in noise, defined as unwanted sound, should not be allowed to continue.

Environmental noise affects many aspects of our lives. Hearing loss and its causes (a major one being noise exposure) constitute a serious public health problem requiring solutions and public controls (Bergman, 1972). According to Kryter (1973), damage risk to the hearing of people exposed to noise is underestimated in some research papers. The noise problem remains a mystery, however, because the extent of each factor involved is not understood adequately to allow comprehension of the total effect of sound exposure upon humans and animals (Lipscomb, 1974). It is important, therefore, to identify detrimental effects of noise, to devise measures of it well correlated with these effects and to establish quantitative relationships between these effects and the measured values of noise exposure (Shaw, 1975).

Miller (1974) concluded in addition to causing hearing loss, noise can (1) interfere with speech and perception of other auditory signals, (2) disturb sleep, (3) be annoying, and (4) interfere with performance of complicated tasks, especially when speech communication or response to auditory signals is demanded. Additional physiologic responses to sound have been demonstrated, including three transient general responses: (1) a fast response of voluntary musculature mediated by the somatic nervous system, (2) slightly slower responses of smooth muscles and glands mediated by the autonomic nervous system, and (3) even slower responses of the neuroendocrine system. Almost certainly a high level of noise can act as a stressor and, for some animals, lead to physiologic changes associated with the general adaptation syndrome (Miller, 1974).

The general adaptation syndrome, as theorized by Selye (1956, 1973), represents the overall response of an animal to a continuous stressor. It occurs in three phases: (1) alarm reaction, (2) stage of resistance (adaptation), and (3) stage of exhaustion. The alarm reaction is characterized by considerable activity of the animal's defensive mechanisms, leading to various observable physiologic changes. If the alarm reaction is not severe enough to kill the animal, the stage of resistance follows, in which some degree of adaptation usually occurs. However, adaptation may result in lowered resistance to infection. Various derangements of the general adaptation syndrome lead to wear-and-tear diseases, the so-called

diseases of adaptation.

Accepting the hypothesis that stress may lower resistance to infection, rats were exposed in the present study to a daily intermittent acoustic stimulus with an intensity of 96 dB(A). As indicators of alterations in these animals' mechanisms of resistance to infection, humoral antibody synthesis and total and differential leukocyte counts were compared between sound-exposed and control animals. Weight gains were also recorded as possible indicators of stress.

Physiologic stress responses to environmental noise normally present are often the result primarily of interactions between specific behavioral activities and noise rather than noise *per se*. Therefore, Kryter (1972) cautioned research on effects of noise upon lower animals usually cannot be generalized to humans. He expressed also the opinion rodents and rabbits should not be used because some of these animals are susceptible to audiogenic seizures. However, rats were used in the present investigation because of their availability, low cost, ease of handling, access to good research facilities for these animals, and in preliminary studies using this level of sound no rats (N= 100+) displayed audiogenic seizures.

Numerous studies in our laboratory indicated high intensity sound affected physiologically animals (rats and chicks) subjected to this trauma (Fell, 1974; Fell, Ellis and Griffith, 1976; Thome, personal communication; Kagan, Ellis and Griffith, 1975, personal communication). However, these effects did not include leukocyte count or immunity reactions. To explore possible ramifications of this type of insult on these parameters, the current study was begun. This report is offered even though its results are largely negative as a guide to further studies.

MATERIALS AND METHODS

Thirty-nine female Long-Evans hooded rats were used, 20 for a 1 week study and 19 for a 4-week study. The 1-week study included 10 control animals (53-56 days old; 150-197 grams, $\bar{X} = 176.5$, SE = 4.3) and 10 experimental animals (54-55 days old; 167-195 grams, $\bar{X} = 176.9$, SE = 2.8). The 4-week study included 10 control animals (58-61 days old; 175-200 grams, $\bar{X} = 186.6$, SE = 2.4) and 9 experimental animals (59-60 days old; 169-196 grams, $\bar{X} = 183.2$, SE = 3.0). Wayne Lab Blox and tap water were provided *ad libitum*.

Animals were housed in two walk-in chambers (7' x 12' x 10') maintained at 83°F ± 5. A 12-hour photoperiod was maintained in each chamber from 0900 to 2100 hours. The sound of the ventilating fan contributed to the ambient noise level of 63 dB(A) ± 3 in each chamber.

In the experimental chamber, a signal of 1.0 Khz at a level of 96

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dB(A) ± 4 was provided daily from 1100 to 1900 hours during the light portion of the photocycle. This sound was presented during 15-minute periods which alternated with 15 minutes of ambient noise. The frequency was generated by a General Radio Oscillator (type 1310-A). Sound levels were monitored every 2 weeks with a General Radio Sound-Level Meter (type 1551-C).

The 1-week and 4-week studies ran simultaneously; the 1-week one commenced 9 days after the 4-week one. For each, animals were allocated randomly into four groups of five. Groups 1 and 4 were control groups and groups 2 and 3 experimental groups.

Data from each group were collected during a 4-day cycle. Data on the five animals of group 1 were collected on day 1 of a cycle, those for group 2 animals on day 2, for group 3 animals on day 3 and for group 4 animals on day 4 throughout the experiment. The 1-week study consisted of 12 cycles and the 4-week study 16 cycles. Individual animals were weighed during all cycles after time was allowed for the animals to acclimatize to the chambers.

Additional procedures included:

1-week study cycle	4-week study cycle	Procedure
5	4	Blood drawn (leukocyte counts and differentials). Experimentals initially into sound chamber.
7	11	Blood drawn (leukocyte counts and differentials; antibody titering). Antigen injection I the following morning.
8	12	Antigen injection II.
9	13	Antigen injection III.
10	14	Blood drawn (antibody titering).
11	15	Blood drawn (antibody titering).
12	16	Blood drawn (leukocyte counts and differentials; antibody titering).

Experimental animals, housed exactly as their respective controls, were exposed to sound for the remainder of the experiment. The terms "1-week study" and "4-week study" were coined because their major chronologic difference is the time between the first and second bleedings, 8 and 27 days respectively. All work was done during the 2 hours of ambient noise either before or after the sound exposure period. On most days, chambers were entered both in the morning and evening. Most work was done on the 1-week animals in the evening and on the 4-week animals in the morning. However, blood for leukocyte values was collected in the evening, commencing at the same time for the three bleedings of each study.

The antigen was a 0.5% suspension of modified rat erythrocytes in phosphate-buffered saline. The cells were fixed with glutaraldehyde by a modified method of Bing et al. (1967) and were sensitized with *Serratia marcescens* lipopolysaccharide W according to the Hoffman (1974) method. Animals under ether anesthesia were injected intraperitoneally according to the following schedule:

Injection	Antigen dosage
I	3.0 ml/kg body weight
II	3.5 ml/kg body weight
III	4.5 ml/kg body weight

Anesthetized animals were bled from their tail tips. Leukocyte differentials (100 cell) were recorded from each of three slides made from individual animals at each bleeding time. For total leukocyte counts, one-half milliliter of blood was added to an equal amount of EDTA-saline solution (2 mg EDTA/ml saline). From this dilution, counts were recorded on a Coulter F automatic cell counter. Direct results from the counter were multiplied by 2 to give total leukocytes/mm³.

Blood for antibody titering was collected in capillary tubes or small

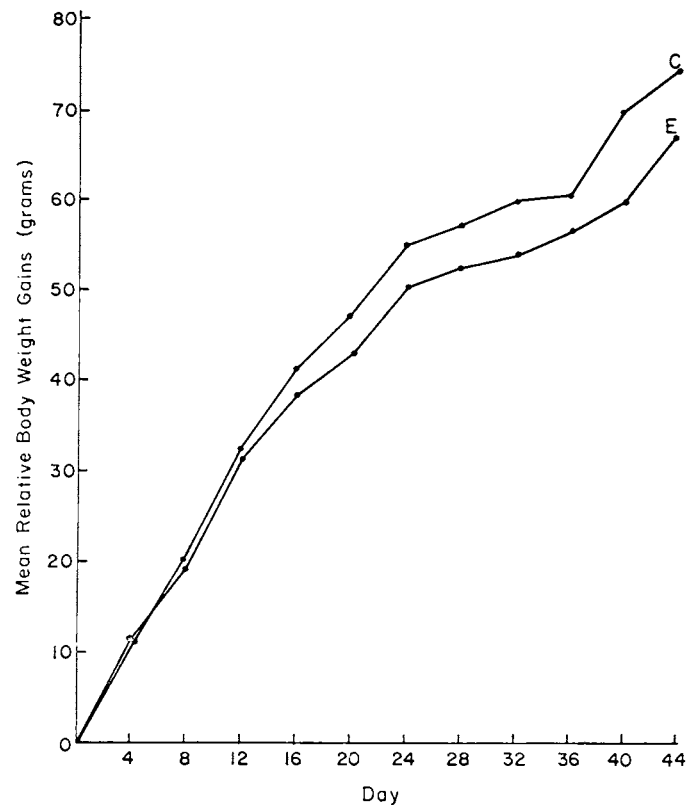


Figure 1. Effect of acoustic stimulation upon total body growth. Mean relative body weight gains (grams) of 1-week animals. All weights recorded between 1900 and 2100 hours. C — Control animals (N = 10). E — Experimental animals (N = 10). Day 16 — After weighing, blood drawn; experimentals into sound chamber after being bled. Day 24 — After weighing, blood drawn; injected the following morning. Days 28, 32 — After weighing, injected the following morning. Days 36, 40, 44 — After weighing, blood drawn.

test tubes, allowed to clot, centrifuged and the sera separated. Sera were stored at 2°C or at -20°C until they were analyzed. Antibody titers were determined by modifications of combined passive hemagglutination techniques of Field et al. (1970) and Neter et al. (1956). The microtiter method using disposable "V" plates was employed (Koehler, 1975).

RESULTS

Average initial weights for each group were: 1-week controls = 176.5 g, SE = 4.3 g; 1-week experimentals = 176.9 g, SE = 2.8 g; 4-week controls = 186.6 g, SE = 2.4 g; 4-week experimentals = 183.2 g, SE = 3.0 g. Weights of the 4-week experimentals on days 40, 44 and 48 were recorded from only eight animals due to accidental removal of half the tail of one animal during the bleeding procedure on day 39. Because this animal's weight response during the following two cycles may not have been representative, data taken from it on days 40, 44 and 48 were eliminated.

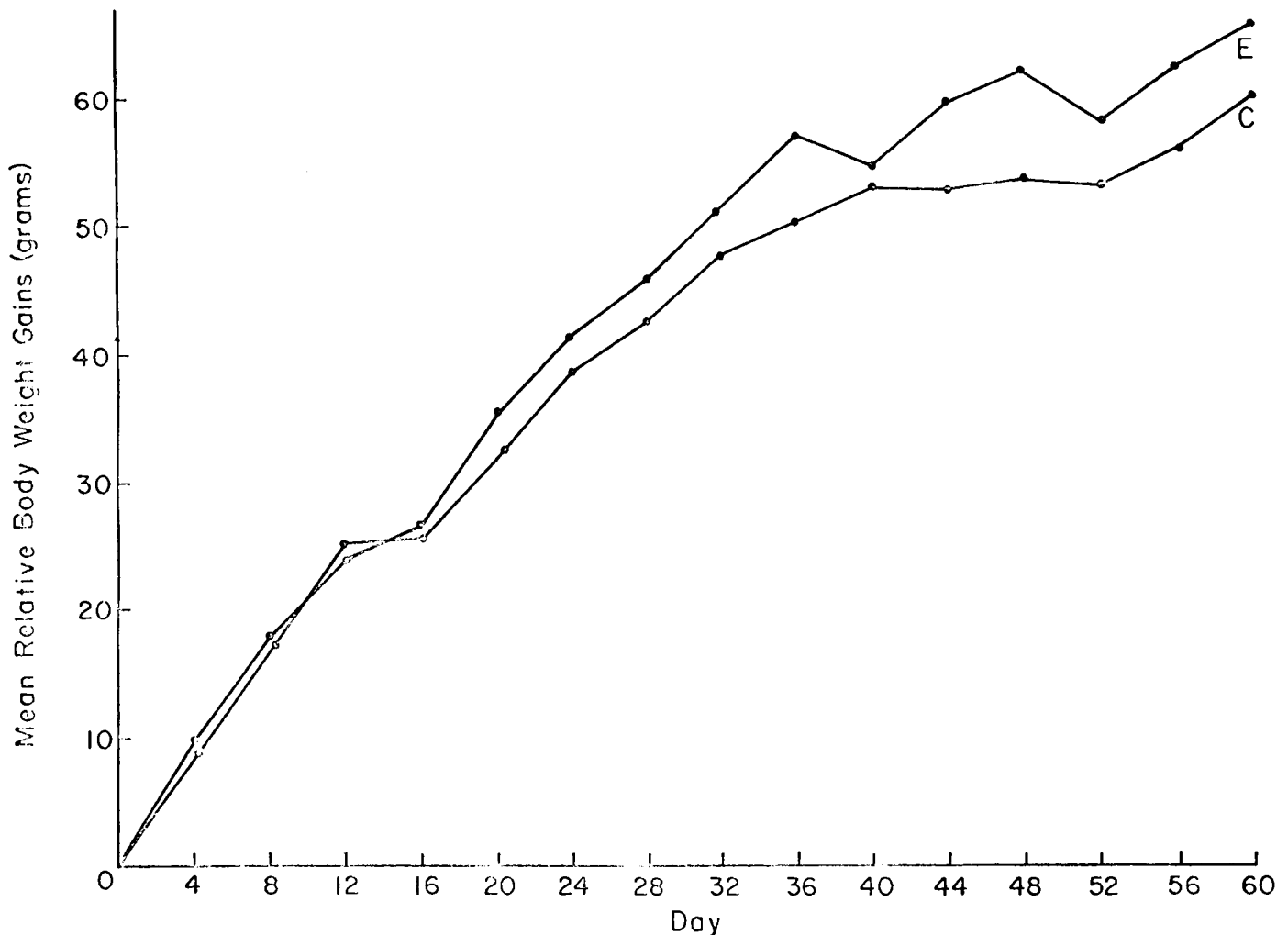


Figure 2. *Effect of acoustic stimulation upon total body growth.* Mean relative body weight gains (grams) of 4-week animals. All weights recorded between 0900 and 1100 hours. C — Control animals (N = 10). E — Experimental animals (N = 9; N = 8 for weight gains recorded days 40, 44 and 48). Day 12 — After weighing, blood drawn (evening); experimentals into sound chamber after being bled. Day 40 — Blood drawn evening before this weighing; after weighing, injected. Days 44, 48 — After weighing, injected. Days 52, 56 — After weighing, blood drawn. Day 60 — After weighing, blood drawn (evening).

Data from each weighing were subjected to a one-way analysis of variance (ANOVA) to calculate an F statistic (Snedecor and Cochran, 1967). During the 1-week study, a slight trend for controls to gain weight faster than experimentals was seen, but no significant differences ($P < 0.05$) between weight gains of the two groups were noted

(Fig. 1). Likewise, during the 4-week study, no significant differences existed between weight gains of the two groups (Fig. 2). In the 4-week study the trend was reversed, with experimentals gaining weight slightly faster than controls.

Animals had natural immunoreactivity to the antigen employed. Their pre-injection titers (\log_2) were: 1-week controls = 1.7, SE = 0.21; 1-week experimentals = 1.2, SE = 0.13; 4-week controls = 1.6, SE = 0.16; 4-week experimentals = 1.6, SE = 0.24 (Koehler, 1975).

One-way ANOVA showed no significant difference between responses of the two groups at each bleeding for each study (Figs. 3, 4). A slight trend for experimentals to respond better than controls was recorded in each study. In the 4-week study, a significant difference was approached ($P < 0.10$) at the third bleeding.

Antibody response obtained was minimal. In preliminary work with albino rats, however, a suspension of heat-killed *Serratia marcescens* and also fixed-sensitized erythrocytes were injected intramuscularly. Responses to these antigens were less than those reported above.

Data (Koehler, 1975) from each bleeding were subjected to a one-way ANOVA and data from each study collectively received a two-way ANOVA to obtain an F statistic (Snedecor and Cochran, 1967). In each study, no significant difference between leukocyte

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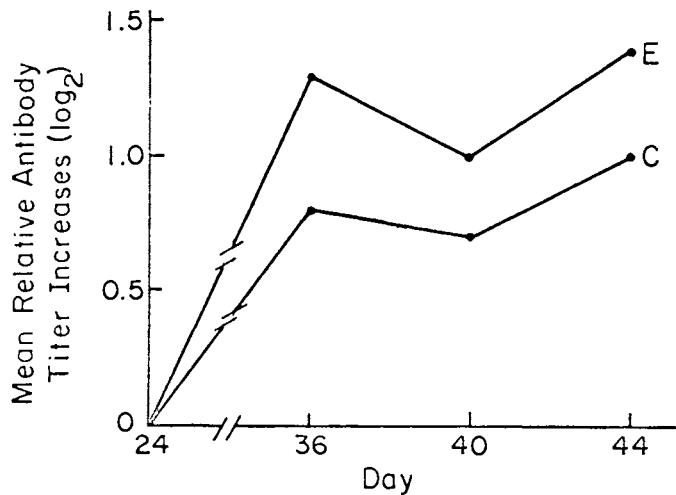


Figure 3. *Effect of acoustic stimulation upon antibody synthesis.* Mean relative antibody titer increases (\log_2) following injection of antigen to 1-week animals. C — Control animals (N = 10). E — Experimental animals (N = 10). Days 24, 36, 40, 44 — Experimentals under sound for 8, 20, 24, 28 days respectively.

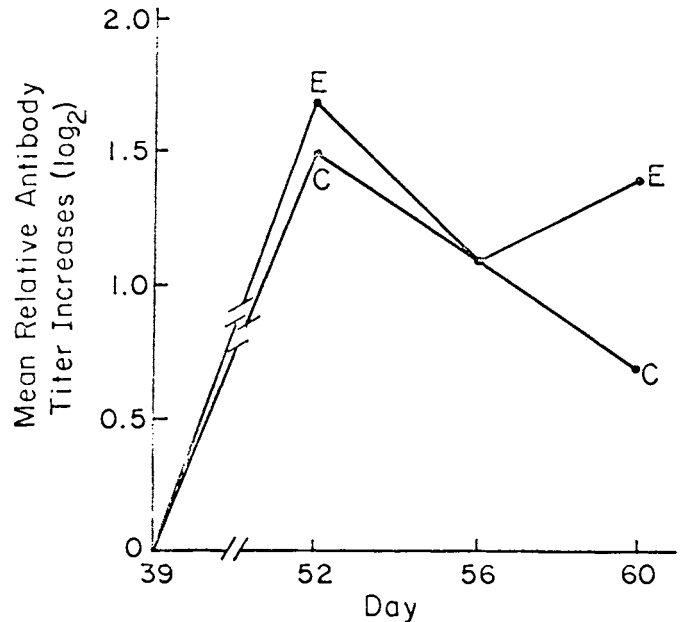


Figure 4. *Effect of acoustic stimulation upon antibody synthesis.* Mean relative antibody titer increases (\log_2) following injection of antigen to 4-week animals. C — Control animals (N = 10). E — Experimental animals (N = 9). Days 39, 52, 56, 60 — Experimentals under sound for 27, 39, 43, 48 days respectively.

counts of controls and experimentals at any one bleeding occurred (Figs. 5, 6). For the 1-week study, the F statistic from the two-way ANOVA approached significance, indicating a trend toward a different treatment and time interaction upon leukocyte counts of experimentals as opposed to controls. Two-way ANOVA of data from the 4-week study showed no significant difference between the two groups.

One-way ANOVA of data for each leukocyte category at each bleeding time and two-way ANOVA of collective data for each cell category were done (Koehler, 1975). For the 1-week study, no significant difference between control and experimental data for any of the categories at any of the bleedings was recorded (Figs. 7, 9, 11). Also, the two-way ANOVA for each of the three cell categories showed no significant difference between the two groups.

Leukocyte differential data for the 4-week study were subjected to one-way and two-way ANOVA. For lymphocytes and monocytes and for neutrophils a significant difference between control and experimental data at the bleeding prior to sound exposure showed, but not later (Figs. 8, 10). Eosinophils showed a highly significant difference ($P < 0.01$) between the two groups at the second bleeding (experimentals under sound for 27 days), but not at the other two times (Fig. 12).

Because of these findings, control and experimental data of the 4-week study were further compared. A one-way ANOVA of quantitative changes from initial values of each cell category at the second and third bleeding times was conducted. For lymphocytes and monocytes, changes at the second bleeding showed no significant difference between the two groups, whereas those at the third bleeding approached significance. For neutrophils, changes at the second bleeding were significantly different. Experimentals showed a greater drop in relative number of neutrophils than controls. At the third bleeding, changes only approached significance. For eosinophils, changes at the second bleeding were also significantly different. Experimentals showed a great increase

while controls showed a slight decrease. At the third bleeding, changes were not significantly different.

Two-way ANOVA of lymphocyte and monocyte and of neutrophil data showed no significant difference between the two groups of the 4-week study, thereby de-emphasizing the significantly different neutrophil changes at the second bleeding. However, two-way ANOVA of eosinophil data showed a highly significant difference in the treatment and time interaction upon eosinophil counts of experimentals in relation to controls. This difference is substantiated by the relative eosinophil count of experimentals which increased greatly and then decreased, while the relative count of controls, initially slightly greater, dropped slightly and then increased greatly.

DISCUSSION

Airborne sound functions primarily as a neurotropic stimulus as opposed to a systemic stimulus such as temperature, epinephrine or histamine (Fortier, 1951). Responses to neurotropic stimuli are manifested via neurohumoral pathways, with the hypothalamus being a major control center. The latter is an important regulator of pituitary gland secretion, so alterations in pituitary hormone output may be part of an organism's response to a particular stimulus.

In the present investigation, acoustic stimulation was ineffective in causing significant differences in body weight gains of female hooded rats. Reduction in weight gain previously noted in animals exposed to noise (Day et al., 1951; Hrubes and Benes, 1965; Vondrakova, 1973; Fell, 1974) could be due to activation of the

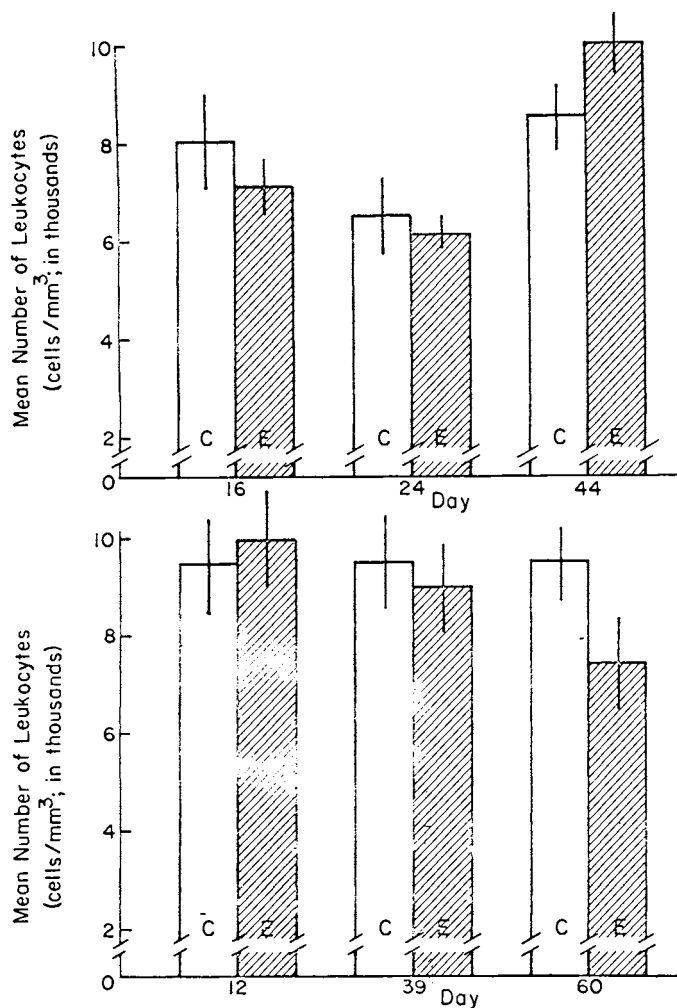


Figure 5. *Effect of acoustic stimulation upon total number of leukocytes in the peripheral circulation.*

Mean number of leukocytes (cells/mm³; in thousands) of 1-week animals.

C — Control animals (N = 10).

E — Experimental animals (N = 10).

Day 16 — Experimentals not yet under sound.

Day 24 — Experimentals under sound for 8 days.

Day 44 — Experimentals under sound for 28 days and all groups have been injected.

Vertical lines represent \pm one standard error of the mean.

Figure 6. *Effect of acoustic stimulation upon total number of leukocytes in the peripheral circulation.*

Mean number of leukocytes (cells/mm³; in thousands) of 4-week animals.

C — Control animals (N = 10).

E — Experimental animals (N = 9).

Day 12 — Experimentals not yet under sound.

Day 39 — Experimentals under sound for 27 days.

Day 60 — Experimentals under sound for 48 days and all groups have been injected.

Vertical lines represent \pm one standard error of the mean.

hypothalamic-pituitary-adrenal axis, resulting in enhanced adrenocortical secretion of glucocorticoids, which decrease protein anabolism. If sound stress is actually capable of decreasing growth hormone release, it could lead to a depressed growth rate. Auditory stimulation reportedly caused reduction in food intake (Sackler et al., 1959; Sackler et al., 1960), so this could be a contributing factor. Apparently none of the above conditions capable of depressing body weight gain was adequately manifested in the within study concerning sound-exposed rats.

Acoustic stimulation did not result in any significant differences in the production of antibodies against antigenically modified erythrocytes in this investigation. Reduced immunologic reactivity resulting from exposure to noise, observed by some (Khaimovich, 1973; Oleshkevich, 1973; Storoshchuk and Veselovskaya, 1967), suggests involvement of glucocorticoids claimed to be immunosuppressive. As Berglund (1952, 1956a, 1956b) demonstrated, optimum time for glucocorticoid administration to reduce primary antibody response probably extends from a few days prior to antigen injection into the period of rapidly rising titers. Therefore, rats in the current project were exposed to sound for 8 or 27 days prior to initial antigen injection. Sound continued for 12 and 13 days, respectively, following the third antigen injection (8 days after initial injection). This time

lapse allowed an increase in glucocorticoid secretion before antigen injection as well as during antibody synthesis.

Stimulating the hypothalamus electrically has resulted in alterations in gamma globulin levels (Fessel and Forsyth, 1963) and antibody titers (Petrovskii, 1961). Stein et al. (1969) found anterior hypothalamic lesions decreased antibody response. They suggested effects of lesions on immune processes may be mediated by a modification of tonic autonomic activity in the sympathetic-parasympathetic nervous system or by changes in neuroendocrine function. The latter mechanism could involve the hypothalamic-pituitary-adrenal axis with subsequent increased glucocorticoid output.

Immunosuppressive effects of adrenocortical steroids may result from their direct effect upon lymphocytes. Lymphatic tissue involution following administration of these hormones reflects their ability to: (1) produce lymphocytokaryorrhexis, (2) inhibit mitosis by destroying cells at metaphase, and (3) inhibit DNA synthesis (Dougherty et al., 1964). Lymphocytokaryorrhexis may account for rapid loss of lymphocytes from all body sites following administration of large doses of adrenocortical steroids. A reduction in number of cells responsible for synthesis of humoral antibody could reflect depressed antibody response. Elliott and Sinclair (1968) correlated

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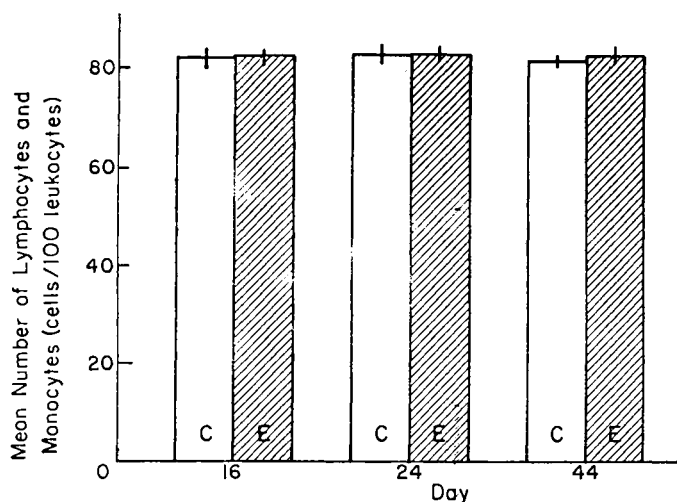


Figure 7. Effect of acoustic stimulation upon relative number of lymphocytes and monocytes in the peripheral circulation.

Mean number of lymphocytes and monocytes (cells/100 leukocytes) of 1-week animals.
 C — Control animals (N = 10; three slides/animal).
 E — Experimental animals (N = 10; three slides/animal).
 Day 16 — Experimentals not yet under sound.
 Day 24 — Experimentals under sound for 8 days.
 Day 44 — Experimentals under sound for 28 days and all groups have been injected.
 Vertical lines represent \pm one standard error of the mean.

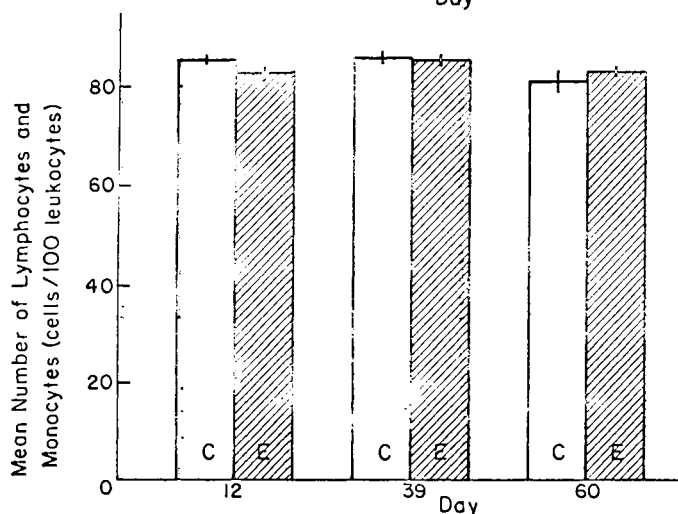


Figure 8. Effect of acoustic stimulation upon relative number of lymphocytes and monocytes in the peripheral circulation.

Mean number of lymphocytes and monocytes (cells/100 leukocytes) of 4-week animals.
 C — Control animals (N = 10; three slides/animal).
 E — Experimental animals (N = 9; three slides/animal).
 Day 12 — Experimentals not yet under sound.
 Day 39 — Experimentals under sound for 27 days.
 Day 60 — Experimentals under sound for 48 days and all groups have been injected.
 Vertical lines represent \pm one standard error of the mean.

7-day hemolysin titers with number of circulating lymphocytes in animals given cortisone acetate. They concluded a major factor in the observed suppression of hemolysin response was depletion of circulating lymphocytes.

Glucocorticoids may act by means other than directly reducing lymphocyte population. They may alter the antibody synthesizing process (McMaster and Franzl, 1961) or phagocytic digestion of antigen may be altered and fail to stimulate antibody formation (Berglund, 1962). The hormones may render lymphocytes and other cells involved in antibody synthesis refractory to an antigen stimulus (Berglund, 1962) or the glucocorticoids may reduce antibody production by their antagonistic effect upon protein synthesis.

Antibody synthesis may be affected if stress-induced thymus involution results in decreased output of certain hormones. Several of the latter have been extracted from the thymus, namely (1) a factor that increased agglutinin production (Milcu and Potop, 1973), (2) fraction B, which enhanced antibody response and blood leukocyte count (Potop and Milcu, 1973), and (3) a lymphocyte-stimulating hormone extract that elevated the number of circulating lymphocytes (Luckey et al., 1973). Such a conclusion is speculative, however, because many effects of thymus extracts have been demonstrated in young animals. A decrease in these hormones may be of no consequence in mature animals.

Growth hormone and thyroxine could be implicated in

immunosuppressive action of sound exposure. Again, higher neurophysiologic control lies in the hypothalamus which is involved in regulation of pituitary output of growth hormone and TSH. The secretion of the latter shows a reciprocal relationship to secretion of ACTH in response to stress (Harris, 1955). Anti-STH antiserum treatment resulted in lymphocyte depletion in lymphoid tissue (Pierpaoli and Sorkin, 1968), perhaps because of decrease in growth hormone concentration. This hormone has an influence upon lymphocyte maturation and an antagonistic action against lympholytic effects of adrenocortical steroids. In addition, reduction in secretion of growth hormone might influence directly antibody production by altering ribosome and messenger RNA synthesis (Pierpaoli et al., 1970).

Hormone production by the thymus may decrease following thyroidectomy (Comsa, 1973). Depressed thyroid gland activity due to sound stress might cause a reduction in antibody production by decreasing thymic hormone output. Although maturation and differentiation of immunocompetent cells continues throughout an animal's life (Pierpaoli et al., 1970), effects of growth hormone and thyroxine upon these processes may be significant only in immature animals. Consequently, these hormones may have little effect upon antibody synthesis in adult life, and their decline during sound exposure may contribute insignificantly to reduction in antibody response.

In this investigation acoustic stimulation resulted in no significant

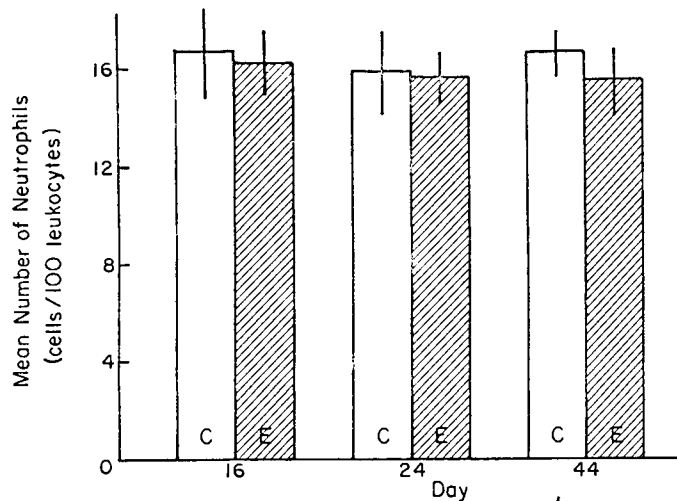


Figure 9. *Effect of acoustic stimulation upon relative number of neutrophils in the peripheral circulation.*
 Mean number of neutrophils (cells/100 leukocytes) of 1-week animals.
 C — Control animals (N = 10; three slides/animal).
 E — Experimental animals (N = 10; three slides/animal).
 Day 16 — Experimentals not yet under sound.
 Day 24 — Experimentals under sound for 8 days.
 Day 44 — Experimentals under sound for 28 days and all groups have been injected.
 Vertical lines represent \pm one standard error of the mean.

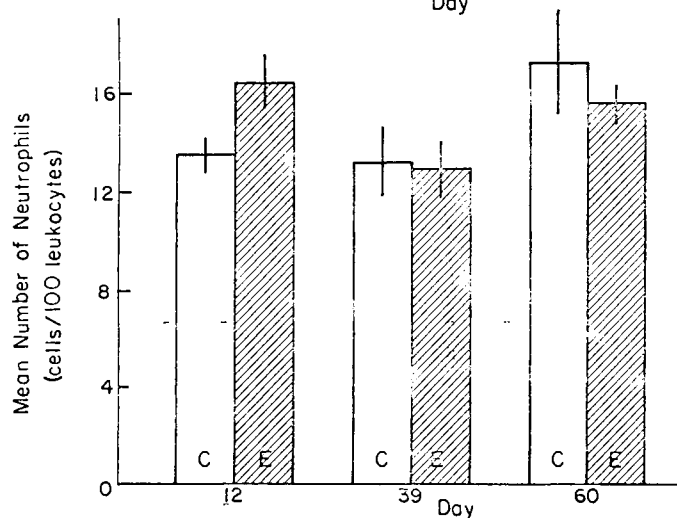


Figure 10. *Effect of acoustic stimulation upon relative number of neutrophils in the peripheral circulation.*
 Mean number of neutrophils (cells/100 leukocytes) of 4-week animals.
 C — Control animals (N = 10; three slides/animal).
 E — Experimental animals (N = 9; three slides/animal).
 Day 12 — Experimentals not yet under sound.
 Day 39 — Experimentals under sound for 27 days.
 Day 60 — Experimentals under sound for 48 days and all groups have been injected.
 Vertical lines represent \pm one standard error of the mean.

differences in total leukocyte counts. Blood samples were collected at the same time for each study to minimize differences caused by the circadian rhythm of circulating leukocytes (Critchlow et al., 1963). Total leukocyte counts were performed prior to sound exposure and again following 8 and 28 days of exposure to the 1-week experimentals and prior to sound exposure and again following 27 and 48 days of exposure to the 4-week experimentals. Chronic responses to stress, however, seem best measured by body weight changes and not by variations in leukocyte counts (Jensen, 1969). Therefore, since no relative weight differences between controls and experimentals were noted at the above times, differences in total leukocyte counts due to the chronic sound exposure would be unexpected.

Adrenocortical steroids seem to be involved in sound-stress leukopenia, as adrenalectomy abolished leukopenia in stressed animals (Jensen, 1969). The lytic effect of adrenocortical steroids upon lymphocytes may be a major factor contributing to leukopenia observed in some sound-exposed animals.

Leukocyte differentials, recorded simultaneously with total leukocyte counts, showed little variation as a result of sound exposure. Values obtained from the 1-week study showed no significant difference between controls and experimentals for any of the three cell

categories, lymphocytes and monocytes, neutrophils and eosinophils. In the 4-week study, analysis of changes from initial values at subsequent bleeding times showed some significant differences between the two groups, however. Changes in relative numbers of neutrophils and eosinophils noted at the second bleeding as well as the two-way ANOVA for eosinophils displayed significant differences.

At the second bleeding of the 4-week study the leukocyte differential count was altered. Relative number of neutrophils decreased more in sound-exposed rats than controls, while relative number of eosinophils increased greatly in experimentals with only a slight decrease in controls. Values from the second bleeding of the 4-week study were obtained just prior to antigen injection, and represented 27 days of sound exposure to experimentals. However, changes in relative numbers of cells observed just prior to antigen injection, which represented only 8 days of sound exposure to the 1-week experimentals, were nearly identical to control values.

A time factor could be implicated in the significant leukocyte differential changes noted above, with the increased length of acoustic stimulation producing the observed differences between controls and experimentals. However, because leukocytes respond rapidly to stimuli, any changes in differential values resulting from sound

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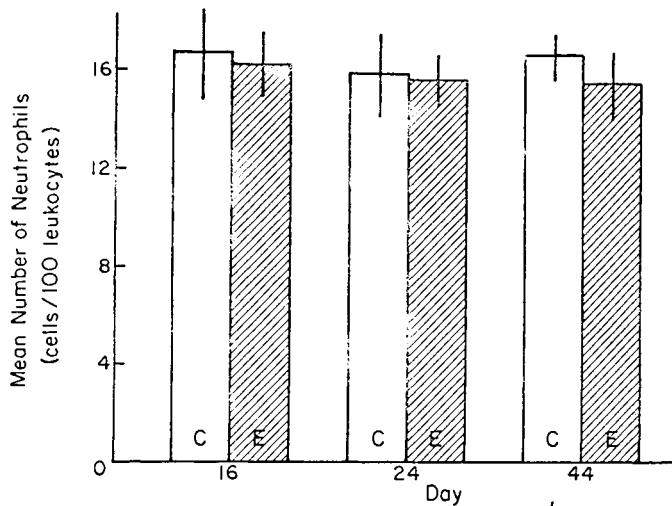


Figure 11. *Effect of acoustic stimulation upon relative number of eosinophils in the peripheral circulation.*

Mean number of eosinophils (cells/100 leukocytes) of 1-week animals.

C — Control animals (N = 10; three slides/animal).

E — Experimental animals (N = 10; three slides/animal).

Day 16 — Experimentals not yet under sound.

Day 24 — Experimentals under sound for 8 days.

Day 44 — Experimentals under sound for 28 days and all groups have been injected.

Vertical lines represent \pm one standard error of the mean.

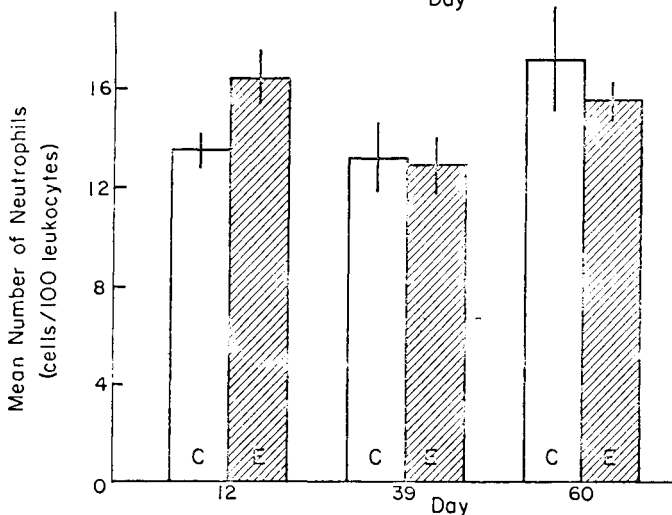


Figure 12. *Effect of acoustic stimulation upon relative number of eosinophils in the peripheral circulation.*

Mean number of eosinophils (cells/100 leukocytes) of 4-week animals.

C — Control animals (N = 10; three slides/animal).

E — Experimental animals (N = 9; three slides/animal).

Day 12 — Experimentals not yet under sound.

Day 39 — Experimentals under sound for 27 days.

Day 60 — Experimentals under sound for 48 days and all groups have been injected.

Vertical lines represent \pm one standard error of the mean.

exposure probably would have appeared by 8 days. Furthermore, changes at the final bleeding of the 4-week study were not different significantly and changes following 28 days of sound exposure to experimentals of the 1-week study showed no differences from control values.

The 4-week experimentals lost weight between days 36 and 40, the latter values having been recorded on the morning following the second bleeding. This temporary weight loss and the significant leukocyte differential changes may have been correlated with the physiologic state of the rats. However, considering other data of this report and those of the literature, these observed changes probably do not represent any sound-exposure effect. These changes seem to be temporary and occurred too late to be changes due to continuing intermittent acoustic stimulation. Some unknown factor must have caused the weight loss and significantly different leukocyte differential changes in the 4-week animals.

In cases of sound-induced quantitative changes in different types of leukocytes, adrenocortical steroids appear to be actively involved. Adrenalectomy in stressed animals reversed eosinopenia of intact-stressed animals (Speirs and Meyer, 1949). Eosinopenic action of ACTH was attributed to agglutination of the cells into major conglomerates consisting of dozens of cells (Makarov, 1971), thereby

producing an effective reduction in number of circulating eosinophils. Eosinopenia followed administration of epinephrine (Fortier, 1951; Speirs and Meyer, 1949), and an increased excretion of this hormone resulted from sound exposure (Ogle and Lockett, 1968; Slob et al., 1973). Epinephrine may enhance secretion of adrenocortical steroids. It may act synergistically with subliminal amounts of these hormones in producing eosinopenia (Recant et al., 1950), or it may act via mechanisms independent of adrenocortical secretion (Jenkins et al., 1953).

No parameter measured in this project was affected by acoustic stimulation to any marked degree. Analysis of weight gains probably indicated whether or not the rats were chronically stressed, since body weights present a general systemic condition and were recorded every 4 days throughout the experiment. Humoral antibody synthesis may be less susceptible than cell-mediated immunity to the effects of adverse stimuli. Depressed antibody production in response to adrenocortical steroid administration may be more of a pharmacologic than a physiologic response, considering the abnormally high doses and non-naturally occurring compounds used in some experiments. Leukocyte values in non-stressed animals are variable normally and change quickly in response to certain demands. Analysis of leukocyte values after many days of sound exposure was probably inappropriate,

since the cells could have demonstrated quantitative changes due to the continuing exposure and already returned to or toward normal. Etherization of the rats for collecting blood samples may have skewed both control and experimental leukocyte values.

Six explanations for lack of positive data may be:

- (1) Experimental sound level, 96 dB(A) \pm 4, was not intense enough to cause marked physiologic deviations. Unrealistically high levels of sound, such as 130-135 decibels (Henkin and Knigge, 1963), were sometimes used by investigators to produce physiologic effects. On the other hand, such responses to sound of less than 96 dB(A) intensity have been reported (Fell, 1974; Thome, 1975, personal communication).
- (2) Sounds with special meaning to the experimental animals, such as those of a predator, or environmental noise with a variety of intensities and frequencies, are probably more efficient in evoking physiologic responses than the monotonous 1.0 KHz signal used herein.
- (3) Long-Evans hooded rats, known for their resistance to respiratory infections, may be able to cope with adverse environmental stimuli better than mice or albino rats used in other studies.
- (4) Hormones peculiar to female Long-Evans rats may have masked any response to the stimulus used in this investigation.
- (5) Even though characterized by hardiness, hooded rats appeared to be adversely affected by the rigorous treatment (excluding sound exposure) of this investigation. Both control and experimental rats were handled frequently and were anesthetized with ether each time they were bled from the tail or injected with antigen. Prior to the fourth and fifth weighings of the 4-week and 1-week studies, respectively, all rats were handled only when weighed. If these early body weight data are extrapolated to the end of each respective study, final values much greater than those actually observed are realized. This finding may indicate both controls and experimentals were significantly stressed, with potential effects of the sound stimulus possibly being masked or inhibited by responses due to the inherent stress of additional procedures.
- (6) Experimental rats may have produced an increased secretion of adrenocortical steroids, particularly during and after the sound exposure periods of the first few days. Increase in adrenocortical hormone secretion in response to noxious stimuli is characteristic of the alarm reaction of the general adaptation syndrome (Selye, 1956; 1973). However, Selye claimed during the period of adaptation the adrenal cortex becomes rich in secretory granules, presumably indicating a return of adrenocortical secretion toward normal. Kryter (1972) reviewed pertinent experiments and concluded during continued exposure to impulsive or steady-state noise, provided it connotes no harmful environmental condition, animals will adapt more or less completely and cease to show arousal responses. If the acoustic stimulus of the present investigation resulted in greater secretion of adrenocortical steroids in experimentals as opposed to controls, the exposed rats must have adapted to the sound rather well, or their increase in hormone output was not very substantial, since none of the parameters potentially susceptible to glucocorticoid action was markedly affected.

SUMMARY

This investigation examined physiologic effects of an acoustic stimulus, 1.0 KHz at 96 dB(A), presented as a 15-minute signal alternating with 15 minutes of ambient noise.

Female hooded rats were exposed to this sequence 8 hours daily for 28 days in one study and for 48 days in another study.

In either study, relative body weight gains recorded throughout and antibody synthesis in response to antigenically modified erythrocytes were not significantly different in sound-exposed animals.

Total leukocyte counts were not significantly different in either study nor did leukocyte differential counts in the shorter study show any differences between the two groups.

At one analysis time in the longer study, significantly different changes in the leukocyte differential count of sound-exposed animals were noted, but these differences were not attributed to any acoustic effect.

Failure of experimental animals to show responses could have resulted from: (1) an inadequate stimulus, (2) sound had no special significance to the rats, (3) Long-Evans rats resist physiologic changes caused by auditory insult, (4) sex hormones masking responses, (5) handling stress masked responses, (6) adaptation to the sound.

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