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Life Span and Organ Pathology in Rats After Life-Long Noise Exposure

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The role of ambient sound level in longevity, cause of death, and incidence of disease was investigated in rats. Two hundred twenty-seven rats (154 Sprague-Dawley (N), 73 spontaneously hypertensive Wistar (SH)), were housed in three sound isolated boxes and subjected to controlled acoustic milieus from approximately three months of age until their natural death. Thirty-nine of the N rats were kept as external controls in a regular animal room. In one of the boxes, no noise was introduced (control). In the other two, the rats were exposed, respectively, to a frequency-modulated, chopped noise with an equivalent level of 80 dB L_{eq} and 100 dB L_{eq}, ten hours daily. It was found that the SH animals had a shorter life span and a higher incidence of cardiovascular disease than the N rats but a lower rate of malignant tumors. No measurable effects on life span or total incidence of disease were seen. Minor differences in incidence of various disease entities were observed but were not consistent across groups. The results do not support the hypothesis that physical sound environment represents a significant cause of somatic disease.

Key words: noise, pathology, life span, rat, cardiovascular disease, tumor

INTRODUCTION

Noise has often been considered an insidious threat to health, both for man and animals [Welch and Welch, 1970; Algers et al, 1978]. It is well documented that noise *can* cause short-term changes in the cardiovascular and endocrine systems (for a recent review, see Borg [1981]). Some evidence from animal experiments shows that long-term noise exposure can cause a permanent, although moderate, rise in blood pressure after exposure over the span of a few weeks to several months [Rothlin et al, 1956; Buckley and Smookler, 1970; Smookler et al, 1973]. Increased weight of heart muscle and adrenal glands has also been observed [Geber and Anderson, 1967; Ising et al, 1974] as well as an increased risk of abortions and fetopathies in animals [Geber, 1973]. Nevertheless, in humans, no direct evidence for a pathogenic effect of noise exists, even though

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several epidemiologic studies point to a higher incidence of symptoms, eg, in the cardiovascular system of workers in noisy industries [Jansen, 1959; Cohen, 1976].

The primary goal of previous animal studies has been to induce "neurogenic" hypertension or "neurogenic" endocrine changes. The exposure conditions have been "nonphysiologic" in several respects, eg, no consideration of the diurnal rhythm and auditory sensitivity of the animal has been taken. Other neurogenic factors such as light flashes and vibration have been added, without usually including or investigating comparable control groups. Exposure situations more similar to the human industrial noise enviroment have not been investigated in model experiments on animals.

The aim of the present study was to investigate the effects of life-long exposure to a noise environment simulating an industrial milieu. The life span and incidence of pathological organ changes were established in a group of normotensive rats and in a risk group of spontaneously hypertensive animals. Short-term and long-term changes of cardiovascular functions have been concomittantly observed and presented elsewhere [Borg, 1980, 1981; Borg and Møller, 1978].

MATERIALS AND METHODS

Materials

The experiments were performed on a total of 227 albino rats; 154 normotensive Sprague-Dawley (N) animals and 73 Wistar rats with spontaneous hypertension (SH) (Okamoto [1969]; delivered by Møllergaards Aulslaboratorier, Denmark, generation F36–37). They constituted three main groups, each housed throughout their lifes in one of three sound isolated boxes from the age of three to four months until their natural death at two to three years of age, and in addition a group of external controls (39 N rats), see Table 1.

Sound Exposure

The three boxes were sound isolated and ventilated to constitute three separate milieus. In box I, the control environment, no external sound was introduced, and the background level, generated by the animals themselves, was about 50 dB (A). In box II the animals were exposed to noise reaching 85 dB SPL (sound pressure level, re. 20 μPa) in the range of 5–15 kHz and in box III to a noise reaching 105 dB SPL (corresponding to an equivalent level of 80 dB L_{eq} and 100 dB L_{eq}, respectively). The exposure sound has been described in detail elsewhere [Borg, 1981]. It consisted of a frequency-modulated narrow-band noise of a width of 1,680 Hz, sweeping from 3.0 to 30.0 kHz at a rate of 0.5 Hz. It was interrupted with a frequency close to 0.5 Hz, ie, with a duty cycle of 50%. The amplitude and frequency modulations were not phase locked, making the microstructure of the sound continuously variable. A timer controlled the exposure by a mechanical switch and delivered the sound for ten hours between 8 PM and 8 AM. A total of two hours of pauses was randomly introduced simulating natural breaks. A fourth group of Sprague-Dawley (N) rats was kept in the department's regular animal room (external control group, environment IV) and routinely cared for by the personnel in the animal department.

Care

The animals were kept in wire mesh cages, maximum five to a cage. When animals died new individuals were not introduced into the same cage, but new groups were continuously added to the study, and all 227 animals were not investigated simultan-

eously. The boxes were ventilated, maintaining the temperature between 22 and 24°C. The animals were kept on a normal night-day schedule with darkness between 8 PM and 8 AM. Food and water were supplied ad libidum. Routine care was given once a week, but otherwise, the animals were minimally disturbed. The sound boxes were inspected for diseased or dead animals each morning.

Autopsy

All animals were autopsied as soon as possible after their natural death. The body and organs were carefully inspected and gross lesions were noted. The weight of the body, heart, liver, kidneys, spleen, adrenals and ovaries or testis was recorded.

Microscopic Analyses

Representative specimens were taken from heart, lung, liver, kidney, adrenal, spleen, stomach, aorta, ovary/testis, brain, pituitary gland, and thyroid gland and from lesions in other organs. The specimens were fixed in 10% formaldehyde solution, sectioned and stained with hematoxylin and eosin, van Gieson's stain, and van Gieson's stain combined with elastin. Frozen sections were stained with scarlet red and hemalun.

Statistical Analyses

Nonparametric statistics were used since the samples did not usually follow normal distribution, ie, the median test and Kruskal-Wallis test [Siegel, 1956].

RESULTS

Clinical Observations

The animals were not subjected to regular clinical investigations, but numerous clinical signs and symptoms were noted during the daily inspections of the sound boxes. In nearly all the aged SH rats, signs of cardiopulmonary insufficiency, dyspnea, and peripheral edema, foremost in the hindlegs, were seen. Mammary fibroadenomas were frequent in females especially in those of the N strain and in some cases developed to massive size (maximum weight 650 gm). Inanition was observed but in no case excessive obesity. No obvious signs of respiratory infection such as sneezing or coughing were seen nor was any case of chronic respiratory disease (CRD) or middle ear infection diagnosed. Macroscopic hematuria and cutaneous tumors were observed in a few rats. Neurological disturbances with progressive atrophy and paralysis of the hind legs were commonly observed in the old N rats. The inefficient control of hind limb movement resulted in the paws being caught in the wire mesh floor of the cages, which necessitated sacrifice of ten animals. Clinical signs were noted for all environments, and there was no obvious difference between exposed and nonexposed groups.

Life Span and Postmortum Observations in the Control Group

The percentage of surviving rats in box I (control) was determined as a function of age. The number of surviving N males and females decreased gradually, whereas the SH stock decreased more rapidly above 15 months (for males) and 20 months of age (for females). The median life span was significantly shorter for the SH males (18 months) than the N males (23 months, $p < .002$) and for SH males than SH females (22 months, $p < .0002$). No significant difference between N males and N females (26 months) was seen, although there was clearly a tendency towards a shorter life span for

the males ($p < .14$). There was no difference between the N animals in box I and the N animals in the external control environment in the animal department ($p < .4$).

Figure 1 shows the incidence of different types of organ lesions in the control groups, in percentages. Table I shows the corresponding information on the main causes of death. No case of middle ear infection or chronic respiratory disease was detected. It is noteworthy that the pattern differed not only between SH and N animals, but also between males and females. For the SH animals, the cardiovascular diseases dominate: myocardial fibrosis (SH females more than N females, $p < .001$), pulmonary edema and especially notable thromboses of the heart and liver (Fig. 2 A, B). In the N animals, renal lesions, such as tubular nephroses, were common and the main cause of death. Also, the N males had significantly more heart disease than N females ($p < .001$). Benign tumors were more frequent in N females than N males ($p < .01$). The malignant tumors occurred in almost 15% of the nonexposed rats, but the total number was too small for a detailed analysis. However, there was a tendency toward a higher incidence of malignant tumors among N females than N males in sound box I, but not among the external controls. There were no significant differences with respect to gastric ulcers and endocrine pathology.

Effects of Noise

Life span. The curves showing the ratio of surviving animals as a function of age follow each other pretty closely, and the median life span was not found to differ between the exposed or the control groups for either strain of rat. Although tendencies to differences could be seen, they were not consistent across groups.

Organ pathology. The total incidence of diagnosed organ lesions in different animal groups is shown in Figure 3. The SH animals, especially the males, have a higher number of diagnosed organ lesions than the N animals, a difference that is mainly due to a high incidence of cardiovascular disease. On the other hand, the SH rats have a lower incidence of tumors than the N animals. There is no difference between sound environments I, II, III, or IV.

The disease panorama for the four groups of rats (N males; N females; SH males; SH females) is illustrated in Figure 4. The relative incidence of various diagnosed abnormalities is shown as one curve for each acoustic environment, thus establishing a disease profile. There are no significant differences among the noise environments except for the incidence of malignant tumors, being higher for N males in box III than for those in box I and II ($p < .02$). However, the highest incidence of malignant tumors was found in the nonexposed control group (IV) housed in the regular animal department ($p < .003$). There was no difference for females nor for SH rats. The greatest number of tumors was seen in environments III and IV, but due to the generalized increase, identification of specific tumors responsible for this difference was not possible. Malignant tumors were considerably more common in N (38/154) than SH rats (3/73). It should be noted that the SH rats died at a lower age than the N rats. 90% of male SH rats were dead at 20 months of age and 90% of female SH rats were dead at 25 months of age. The mean age of rats dead from cancer was 23 months or similar to the age when most SH rats were already dead.

Organ Weight

No systematic difference related to sound environment was seen.

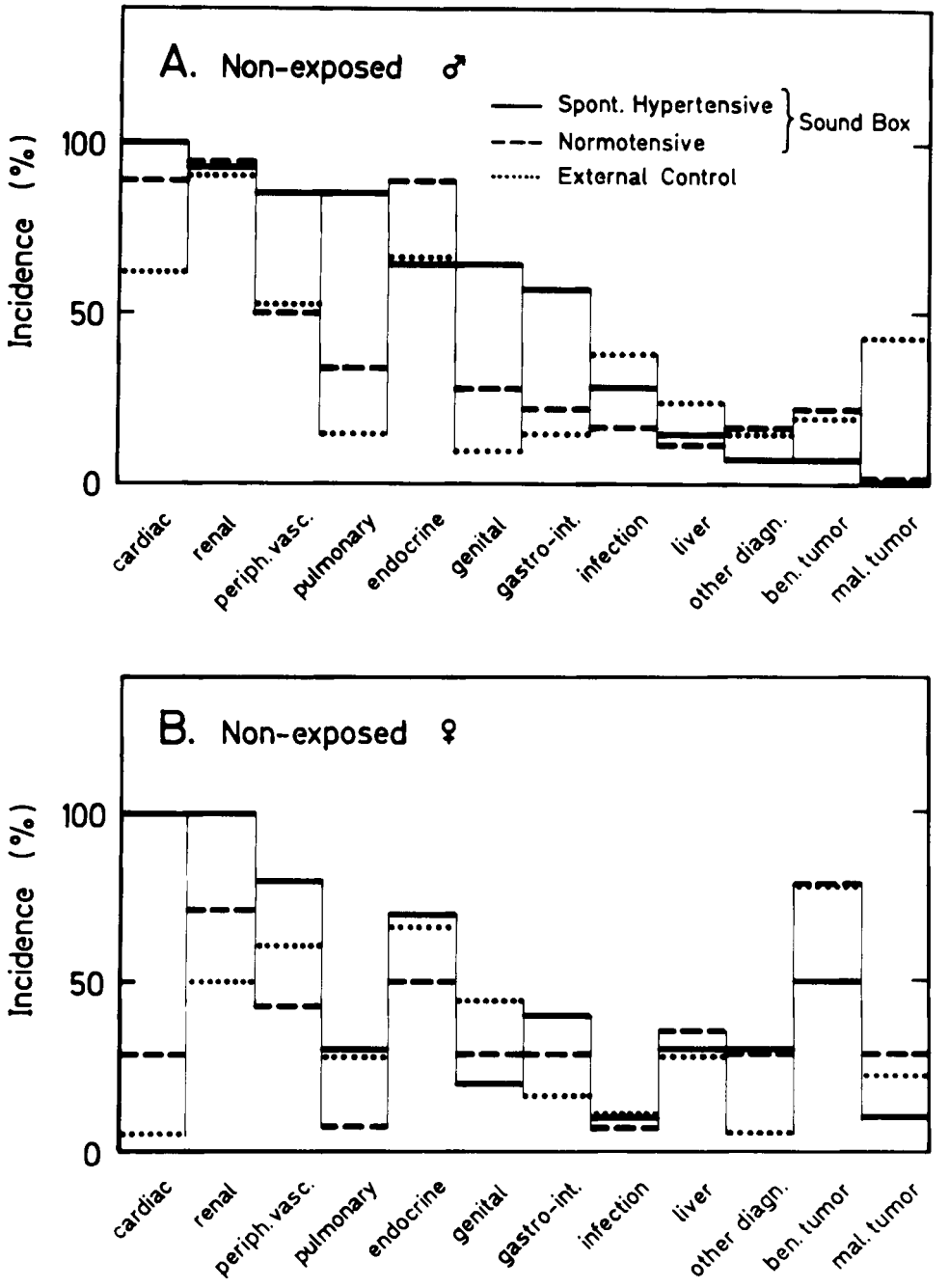

Fig. 1. Incidence of major organ lesions in 95 nonexposed rats. A, males; B, females.

TABLE 1. Main Causes of Death (%) for 227 Rats Living in Four Different Sound Environments From Approximately Three Months Age.

	♂Normotensive				♂Spontaneous hypertensive			♀Normotensive				♀Spontaneous hypertensive		
	I	II	III	IV	I	II	III	I	II	III	IV	I	II	III
No of animals	18	26	18	21	14	7	16	14	25	15	19	10	8	16
Cardiac (%)	11		6		93	100	100			7	6	50	50	76
Peripheral vasc (%)	11		6		7				8			20	25	6
Renal (%)	56	85	50	57				29	40	27	17	10		6
Malign tumors (%)		15	28	19				29	16	7	22		12	
Benign tumors (%)	6			5				21	20	33	39			
Other diagnoses (%)	16		10	19			[a]	21	16	26	16	20	13	12

[a]Four died of overheating.
I: sound isolated box, nonexposed.
II: sound isolated box, 80 dB L_{eq}.
III: sound isolated box, 100 dB L_{eq}.
IV: nonexposed rats in the ordinary animal room.

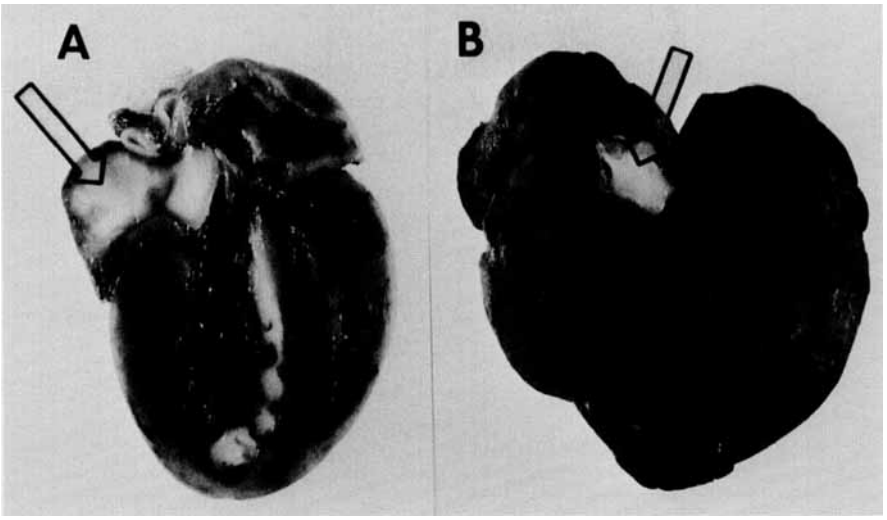

Fig. 2. Thrombosis in heart (A) and liver (B) in spontaneously hypertensive rats with clinical signs of cardiac insufficiency and failure.

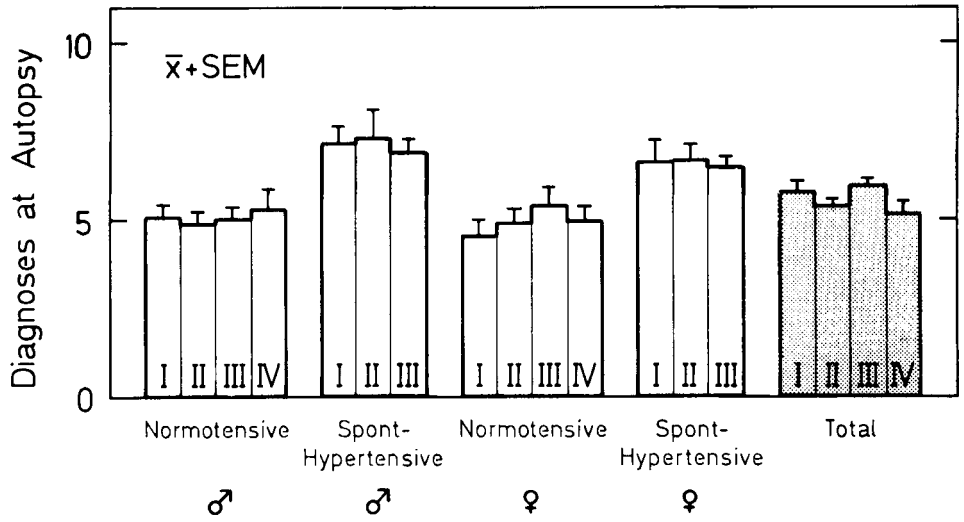

Fig. 3. Mean (+SEM) of number of diagnosed organ lesions at autopsy of 227 rats living in different acoustic environments from three months of age. I: sound isolated box, nonexposed; II: sound isolated box, 80 dB L_{eq}; III: sound isolated box, 100 dB L_{eq}; IV: external controls, ordinary animal room.

DISCUSSION

The results of the present investigation do not support the theory that noise increases the risk for somatic illness. Neither systematic nor significant differences were observed between the exposed and nonexposed normal (N) or risk group (SH) rats. This is in accord with earlier findings showing a lack of long-term effect on blood pressure and other physiological parameters evidenced in the same group of animals [Borg and Møller, 1978; Borg, 1981]. The only significant biological effect of noise exposure to be observed was a hearing loss [Borg, 1979], which was greater for the animals in sound environment III than in II, and greater for the SH than the N animals. The results therefore point to hypertension as a risk factor for noise-induced hearing loss, rather than noise being a risk factor for hypertension [cf. Jonsson and Hansson, 1977].

There was no difference in incidence of the major disease groups among the different sound environments. Only with respect to malignant tumors was a higher disease incidence seen in the noise-exposed animals. This difference, applied to the total data, was found to be caused entirely by the increase in the N male rats. However, highest incidence of tumors was seen in the control group housed in the animal department. The tumor incidence was lower for SH rats than N rats, but this difference must certainly be related to the early death of SH animals from cardiovascular disease. The final conclusions and implications of tumor incidence in noise environments can only be determined after investigation of a much larger number of animals. At present, little, if any, significance should be attached to this difference.

The lack of difference between exposed and nonexposed rats may be due to factors that abnormally shorten the life span or increased the incidence of disease for all animals in the present investigation. Such factors may hide possible pathogenic effects of sound. The life span and incidence of diseased organs is, however, roughly in accordance with that described in earlier studies on nonexposed rats. The high incidence of

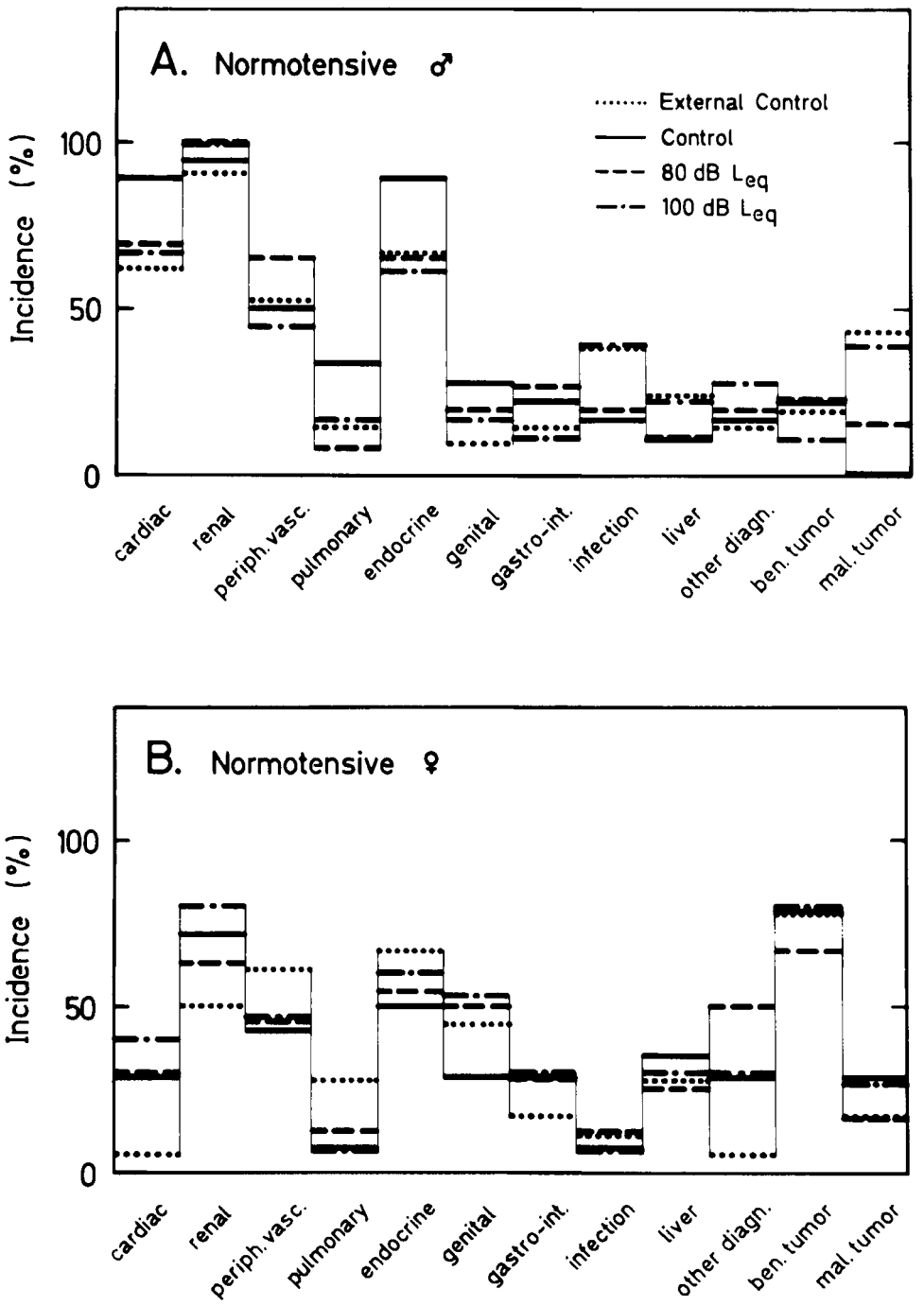
A. Normotensive ♂
External Control
Control
80 dB Leq
100 dB Leq
Incidence (%)
100
50
0
cardiac
renal
periph. vasc.
pulmonary
endocrine
genital
gastro-int.
infection
liver
other diagn.
ben. tumor
mal. tumor
B. Normotensive ♀
Incidence (%)
100
50
0
cardiac
renal
periph. vasc.
pulmonary
endocrine
genital
gastro-int.
infection
liver
other diagn.
ben. tumor
mal. tumor

Figure 4A, B.

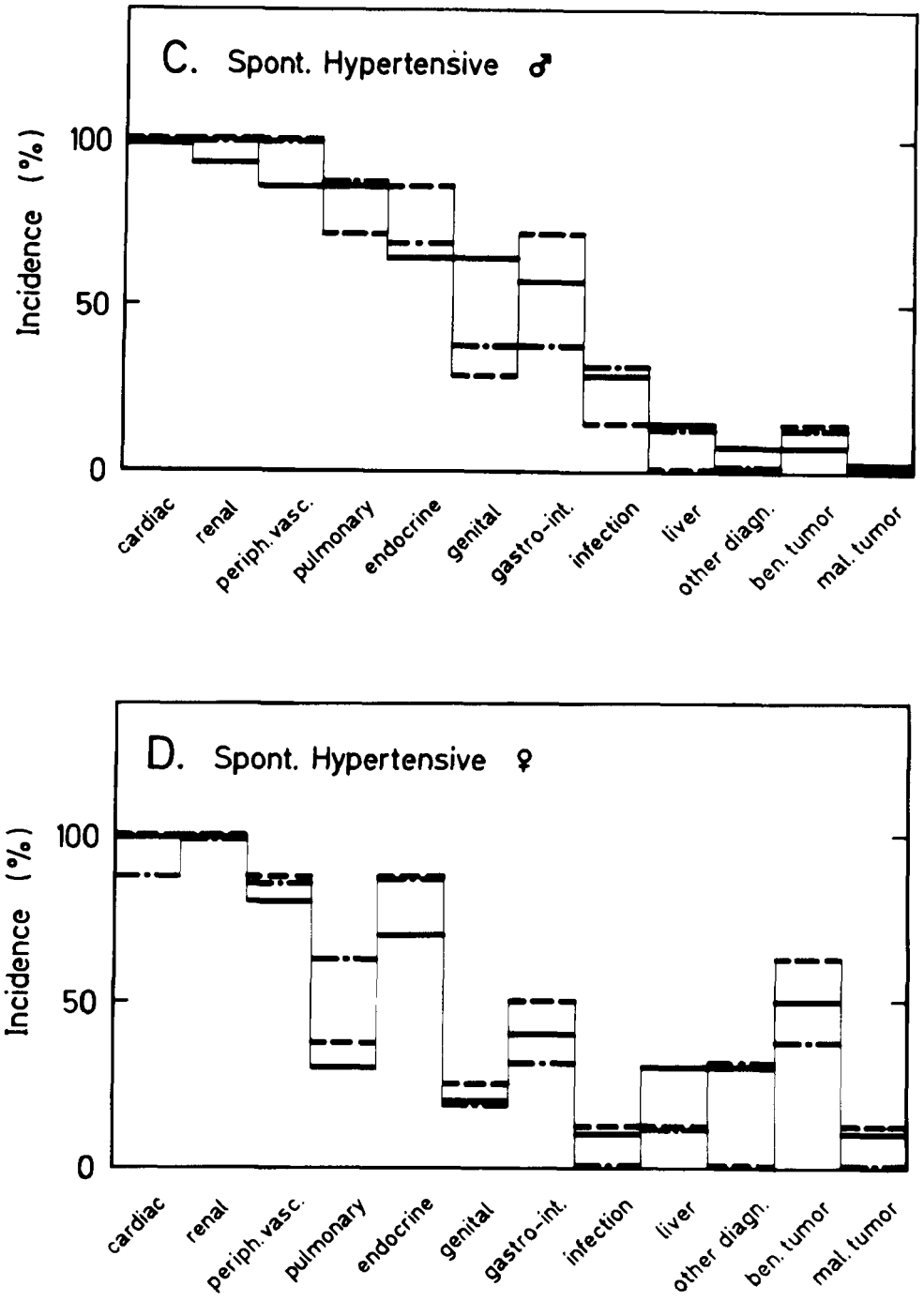

Fig. 4. Incidence of major organ lesions at natural death in 227 rats living in four different sound environments from the age of three months. A, normotensive males; B, normotensive females; C, spontaneously hypertensive males; D, spontaneously hypertensive females.

cardiovascular disease in the SH rats and the incidence of renal disease in the N animals has been pointed out earlier by Okamoto [1969], Berg [1967], and Simms [1967]. The incidence of 12% [Davis et al, 1956] and 10% (MacKenzie et al, 1973] for malignant tumors in autopsy material is in fair accordance with our data.

It is of course difficult trying to apply the results of this model to humans. Human populations naturally contain a much wider range of biological variation than the two groups of rats in this study. Furthermore, psychological aspects, information value, and masking of speech have been intentionally excluded in the present model. Its aim has been to test the hypothesis that short-term reactions seen in response to sound stimuli have a counterpart in long-term physiological alterations and in organ pathology. In spite of these differences, it can be noted that 1) rats have at least as pronounced short-term reactions to sound as humans [Borg, 1980], 2) the sound used in the model experiments was biologically realistic for creating a hearing loss comparable to that seen in industrial environments in humans, 3) the lack of long-term physiological and pathogenic effects is most likely due to habituation [Borg, 1981]. It remains to be shown in further investigations whether the life span and incidence of disease can be altered by factors delaying habituation, eg, information value of components in the acoustic environment.

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