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A STUDY OF THE BIOLOGICAL EFFECTS OF CERTAIN ELF ELECTROMAGNETIC FIELDS

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ABSTRACT

Experiments were performed to detect possible physiological effects of air and simulated earth return electromagnetic fields of 45 and 75 Hz at 100 V/m peak-to-peak (35 V/m rms) on young mice. The criteria used were growth rate, serotonin metabolism and alteration in susceptibility to infection with influenza virus. There were no changes in these three categories.

Introduction

The possibility of utilizing ELF radio signals as a means of communicating with submerged submarines has been investigated for many years by the United States Navy. The concept was proved feasible in 1963 and on the basis of theoretical and experimental studies it was decided to use the earth itself as an integral part of the transmitting antenna. Transmission at ELF (< 100 Hz) is in marked contrast to that of A.M. radio stations which broadcast at 500-1600 x 10^3 Hz. A major characteristic of ELF radio signals is their extremely long wavelength, e.g. 4140 miles at 45 Hz, and this property imposes the need for an antenna many miles long. The considerable problems posed by antenna length and configuration and by power requirements have been solved so that acceptable designs for transmitting and receiving components now are available.

Basically the transmitter antenna will consist of an insulated cable buried in the earth and grounded at either end to produce an earth return circuit. A second antenna will be installed at right angles to the first in order to transmit an omni-directional pattern. Since single cables will require very high currents to achieve a suitable signal output, multiple parallel cables are to be deployed in a grid of some 40 by 80 miles. Analysis of such an array operating at 75 Hz and radiating power of several hundred watts indicates that the electrical field strength in the ground above the ~~feeder~~ antenna lines will ~~by~~ be ca 0.07 V/m rms decreasing with distance from the grid. By way of comparison, an average field strength of 0.09 V/m prevails near the earthed end of an average home electric power system. Comparable measurements near commercial radio and TV transmitting

antennas are several orders of magnitude larger. The magnetic fields above the ~~feeder~~ antenna lines will average ~~0.13~~ 0.2 gauss or 2 orders of magnitude less than that produced by common household appliances, such as electric shavers and blankets.

Although the values are low, one is obliged to consider the possibility that long term exposure conceivably could have a detrimental ecological effect. Until recently no systematic study of this potential hazard had been made. To rectify the lack of information and to provide rational evaluation of the operational biological impact of such exposure, the Navy has sponsored laboratory investigations of the effects of ELF electromagnetic fields on fish, plants, animals and insects. The present paper deals with one phase of these studies: the influence of ELF environment on the growth of mice, their serotonin metabolism and their susceptibility to infection with influenza virus.

Materials and Methods

The experiments described in this paper were conducted in the Air Ion Laboratory at the School of Public Health, University of California, Berkeley, California, and utilized the facilities originally constructed for investigating the biological effects of air ions (Krueger et al., 1962; Krueger and Reed, 1972).

The mice employed were females of the NAMRU-SPF strain, 3 weeks of age. They were fed Purina Lab Chow and water ad libitum and were housed in cages specifically designed to provide air and ground fields simulating those prevailing under full-scale ELF operating conditions (Fig. 1). Construction details follow. Two opposing walls are light gauge aluminum 15.5 cm x 17.2 cm. The other walls are plexiglass 17.2 cm x 20.5 cm. Holes in one plexiglass wall allow access to water and feed held in

extramural containers. The bottom consists of 32 stainless steel rods 3.14 mm in diameter connected in series by 2200 ohm resistors.

Fifteen cages were mounted on shelves in each of two cubicles. The latter were made of 6 mm enameled masonite, measuring 1 m x 1.5 m x 2.1 m and were lighted twelve hours/day with single 75 watt electric bulbs. They were supplied with pollutant-free air at a flow of 3.45 m^3/min. Entering air was passed through a purifying train consisting of a duct heater, a Trion electrostatic precipitator, a Barneby Cheney activated carbon filter, an absolute filter and a Carrier air conditioner. Temperature was maintained at 26°C and humidity averaged 46% R.H. Small air ion levels were measured with a Wesix type ion collector, Royco Power Module and Carey femtoammeter; their concentrations ranged from 100 to 250 positive ions/cm^3 and from 80 to 200 negative ions/cm^3.

The cages were wired in parallel to a Hewlett-Packard audio oscillator Model 201C operating at 45 Hz or 75Hz. At weekly intervals each cage was monitored with a Type 561A Tekronix oscilloscope for constancy of signal input.

In assessing the biological effects of exposure of weanling mice to ELF electromagnetic fields, we studied growth, serotonin metabolism and susceptibility to influenza virus.

1. Rate of growth: Mice were weighed individually at the start of each experiment (Day 0) and twice weekly therafter. The arithmetic means and 95% confidence intervals were calculated for each period.

2. Serotonin metabolism: In some experiments blood levels of serotonin were determined. Thirty lambda blood samples were removed from the tail veins of mice with a micropipette and were analyzed for serotonin by the spectophotofluorometric method of Udenfriend et al. (1955), as modified by Krueger et al. (1963). A uniform sampling schedule was

followed in order to minimize the possible influence of circadian periodicity on the serotonin values.

To determine brain concentrations of serotonin mice were anesthetized with ethyl chloride and exsanguinated from the axillary vessels. Brains were removed, rinsed with distilled water, blotted dry and weighed. This procedure was performed as rapidly as possible so that time elapsing from onset of anesthesia through weighing was < 2.5 minutes. The whole brain was ground in a Konti tissue grinder (size A) in 1 ml of 0.1 NHCl. The brain homogenate was decanted and the grinder rinsed twice with a total volume of 1 ml EA (0.75 gm1-ascorbic acid + 25 ml of 1% EDTA). The homogenate was agitated in a vortex mixer and immediately placed in an ice bath. The extraction of serotonin was carried out by the same method used for blood. After extraction the samples were centrifuged for 5 minutes at 1200 r.p.m.'s. The heptane supernate was carefully aspirated, taking care to remove all the material deposited at the interface. Spectrophotofluorometric assay for serotonin followed the procedure employed for blood. The arithmetic means and 95% confidence intervals were calculated for each group of blood and brain serotonin values.

3. Ssuceptibility to influenza: In 6 experiments the possibility was explored that the resistance to infection with influenza virus had been altered by exposure to ELF electromagnetic fields. For this purpose, high titer egg passage influenzal virus of the PR8 strain was used. One ml aliquots kept at -70°C were thawed as required and dilutions were prepared in phosphate buffered saline pH 7.3. The final dilution was kept in an ice bath and brought to room temperature just before intranasal instillation. The mice were challenged while lightly anesthetized with alcohol-chloroform-ether mixture by delivering 0.05 ml of the diluted virus from a micropipette into the nares. The rates of death were followed and the resulting data

were expressed as cumulative mortality rates or as LD_{50}'s, according to the method of Reed and Muench (1938).

Experimental Results

1. Growth. Two preliminary experiments were performed at 45 Hz and 5 V/m, peak-to-peak (1.75 V/m rms), the first lasting 30 days and the second 36 days. The results were essentially identical and followed the pattern displayed in Figure 2. There was no significant difference in rate of growth between the animals exposed to ELF conditions and the controls.

Subsequently, we conducted two more experiments at 45 Hz, but increased the voltage to 100 V/m. Here again ELF treatment produced no deviation from the normal rate of growth (Fig. 3).

The largest test series involved six experiments at 75 Hz and 100 V/m. The curves displayed in Figure 4 are typical of the results; namely, a lack of any influence of ELF conditions on the growth of mice during periods varying from 12 to 26 days of exposure when growth was most rapid.

2. Serotonin metabolism. Blood and brain levels of serotonin were determined in one experiment performed at 45 Hz and 100 V/m. The values are presented in Table 1 and show no effect due to ELF treatment for 30 days in the case of blood serotonin and for 37 days in the case of brain serotonin.

When ELF conditions of 75 Hz and 100 V/m were imposed, there was no statistically significant difference between the treated and untreated animals in terms of blood and brain serotonin levels (Table 2).

3. Susceptibility to infection with influenza virus. We ran four separate tests at 75 Hz and 100 V/m to determine whether ELF conditions changed the susceptibility of mice to infection with influenza virus. Using the cumulative mortality rates and LD_{50} titers (method of Reed and Muench) as criteria, we found no evidence of altered resistance (Fig. 5).

Discussion

At the Sixth International Biometeorological Congress at Noordwijk, The Netherlands, in 1972, Reiter, chairing the symposium on biological effects of electric, magnetic and electromagnetic fields, summarized the sense of the session as follows:

> "It was clearly shown that significant biological effects of electric, magnetic and electromagnetic fields exist, even if the latter are only of weak strength. On the other hand, the biological effects of these are overshadowed by those of other active factors of our everyday environment. Nevertheless, the effects of electric, magnetic, and electromagnetic fields may, from time to time, be quite important."

It has come to be recognized that the study of such biological effects presents formidable difficulties. In his 1968 review of the subject Tromp states: "One of the principal causes of the many contradictory reports in electro-biometeorology, less often encountered in other fields of science, is the extremely difficult technical character of these studies".

If one accepts the conclusion that a wide range of electric, magnetic and electromagnetic fields are capable of influencing physiological processes, there is valid reason to investigate the environmental impact of the ELF fields developed by the proposed Project Sanguine transmitter, even though many electrical installations and appliances in common use for many years produce magnetic and electric fields equal to or greater than those projected for Project Sanguine. It follows, too, that meticulous care must be exerted in defining experimental conditions.

The experiments reported in this paper were concerned with the effects of fields simulating those created by the Project Sanguine transmitter. The tests were conducted in chambers constructed of enamelled masonite and provided

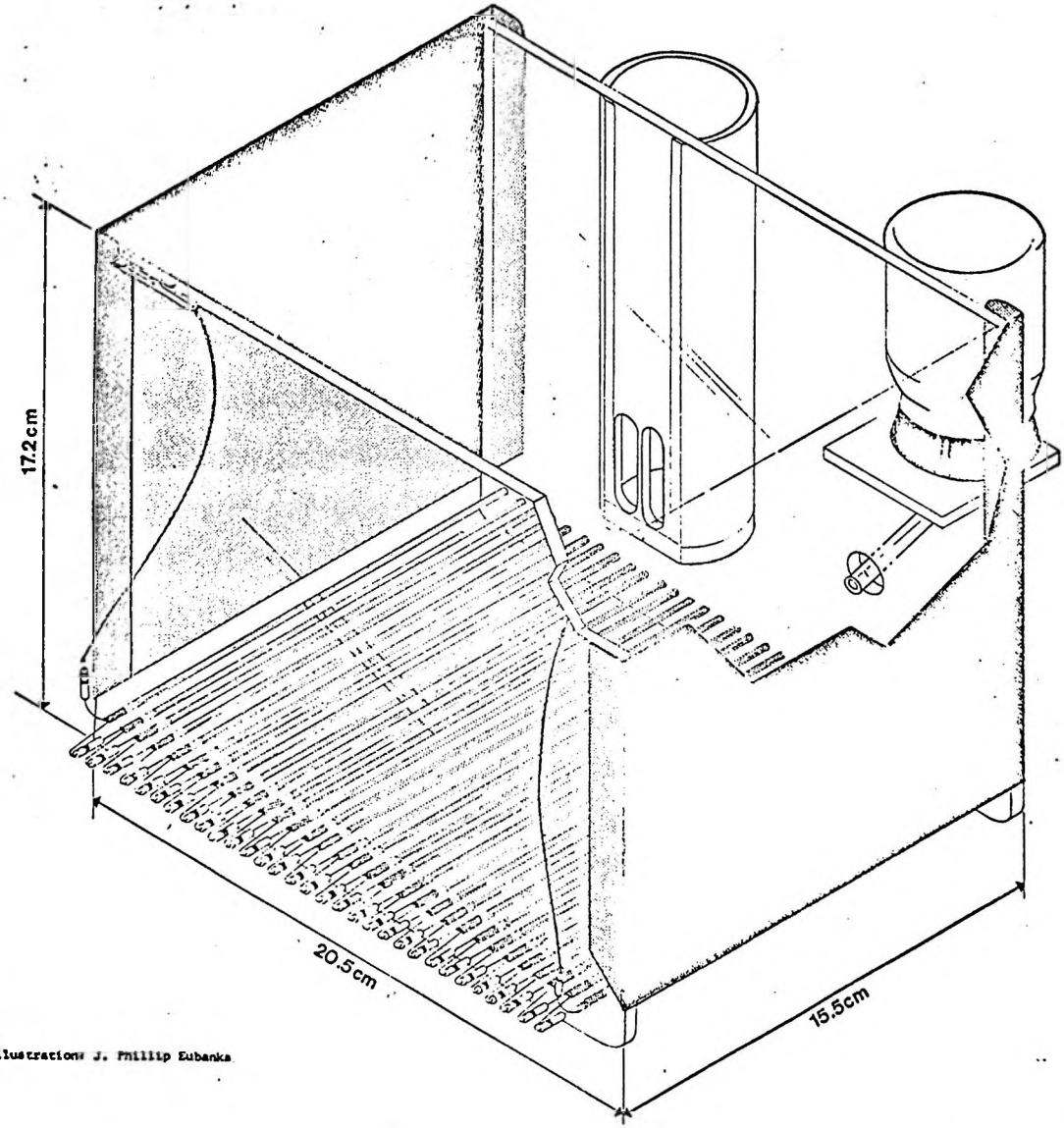
with pollutant-free air maintained at 26°C and an average humidity of 46%. The air ion background was 100-250 small positive ions/cm^3 and 80-200 small negative ions/cm^3. Light was provided for 12 hours per day. The subjects used were young female SPF mice of the NAMRU strain kept in cages so designed that the animals (four per cage) were continually exposed under foot and in the air to electric ~~magnetic~~ fields developed by a current of 45 Hz or 75 Hz at 100 V/m, peak-to-peak (35 V/m rms). Control mice were housed in identical cages which had no current input.

The functions observed for possible effect were growth, serotonin metabolism and resistance to infection with influenza virus. These criteria were chosen because in mice all three respond to minor shifts in the environment, e.g. changes in the charge and numbers of small air ions in the ambient atmosphere (Worden, J. L., 1953; Krueger, A.P. et al., 1966; and Krueger, A.P., 1970). Also, there is good evidence that: 1) atmospheric electrical imbalance--long considered to be of no biological significance--actually is capable of causing illness in weather-sensitive individuals and 2) this effect is mediated by air-ion-induced alterations in serotonin metabolism (Sulman, et al., 1974).

There were no detectable differences between mice kept in the ELF environment and controls as reflected in rates of growth, levels of serotonin in blood and brain or susceptibility to challenge with measured amounts of influenza virus. These findings, of course, do not exhaust all possible phases of ELF biological impact. They indicate, however, that three types of physiological phenomena known to respond to what are considered to be insignificant changes in atmospheric electricity are not influenced by the ELF environment under investigation.

Figure 1.

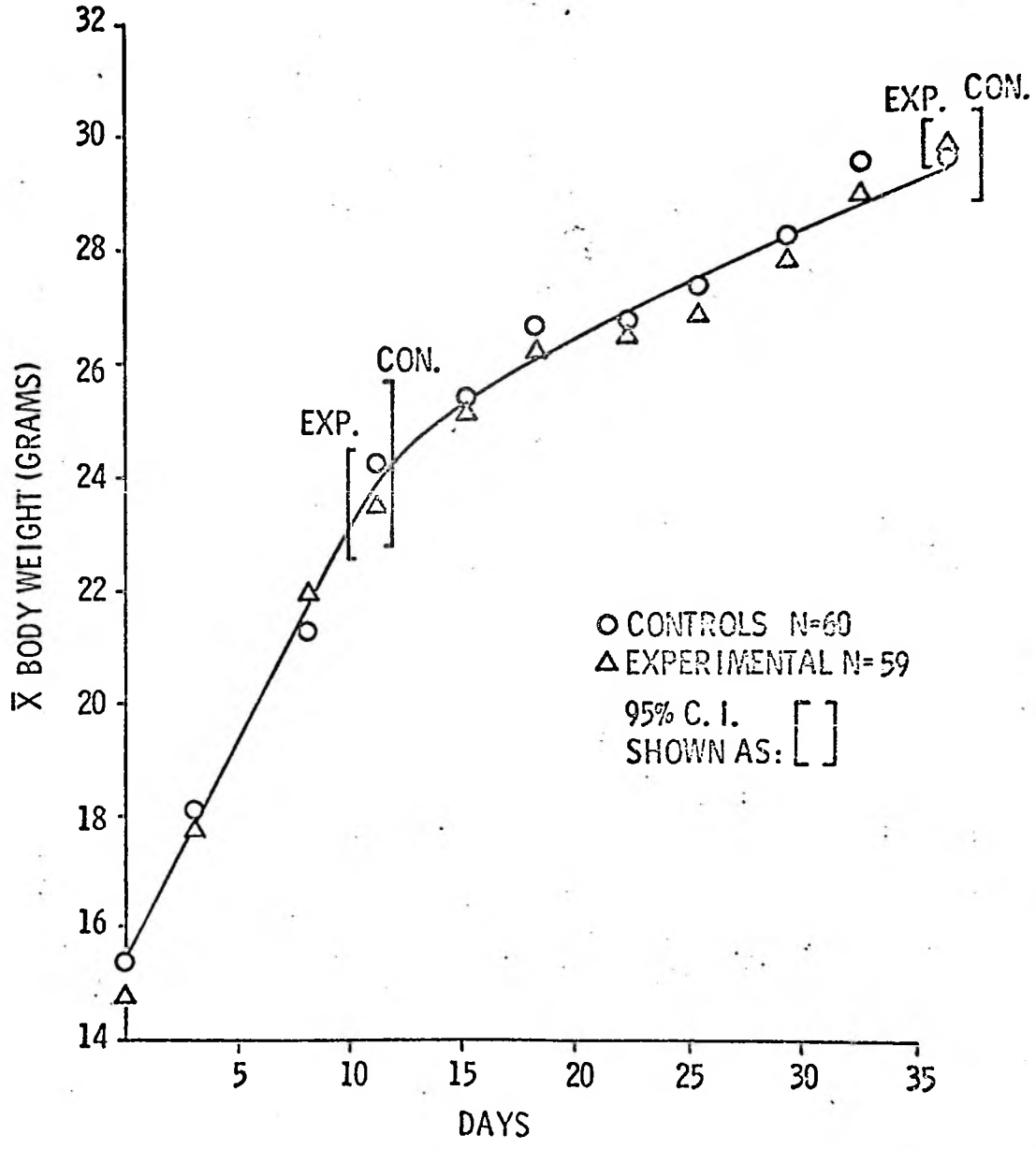
Construction details of cages designed to provide ELF environment. See text for description.



Illustrations: J. Phillip Eubanks

Figure 2.

Growth curves of control and treated mice exposed to ELF environment of 45 Hz at 5 V/m. for 36 days. N=60 at start for each group. One experimental animal was lost.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16
14
X̄ BODY WEIGHT (GRAMS)
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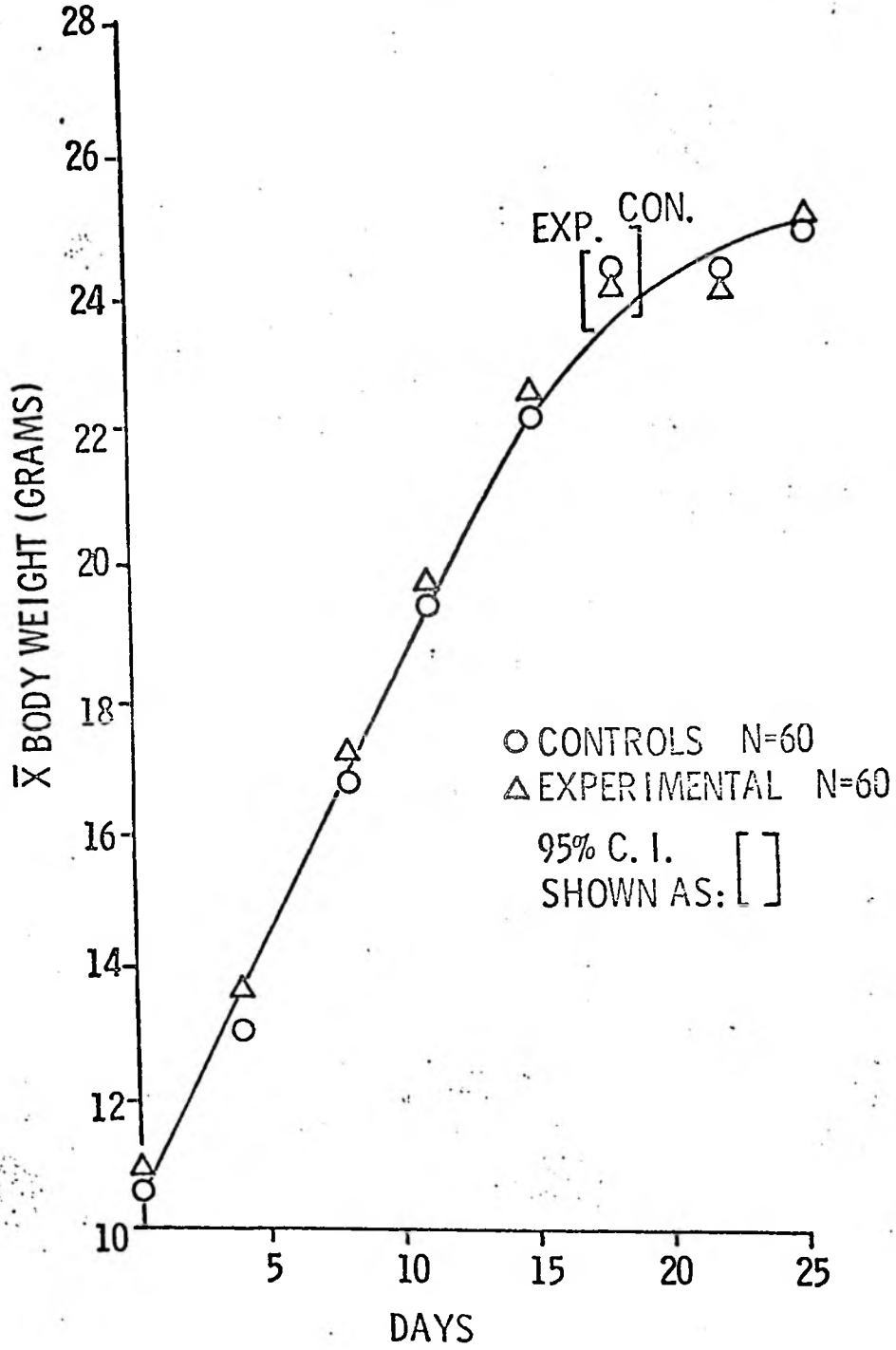
15
20
25
30
35
DAYS
EXP.
CON.
EXP.
CON.
CONTROLS N=60
EXPERIMENTAL N=59
95% C. I.
SHOWN AS: [ ]

Figure 3.

Growth curves of control and treated mice exposed to ELF environment of 45 Hz at 100 V/m for 25 days. N=60 for each group.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$\bar{X}$ BODY WEIGHT (GRAMS)
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CON.
○ CONTROLS N=60
△ EXPERIMENTAL N=60
95% C. I.
SHOWN AS: [ ]
5
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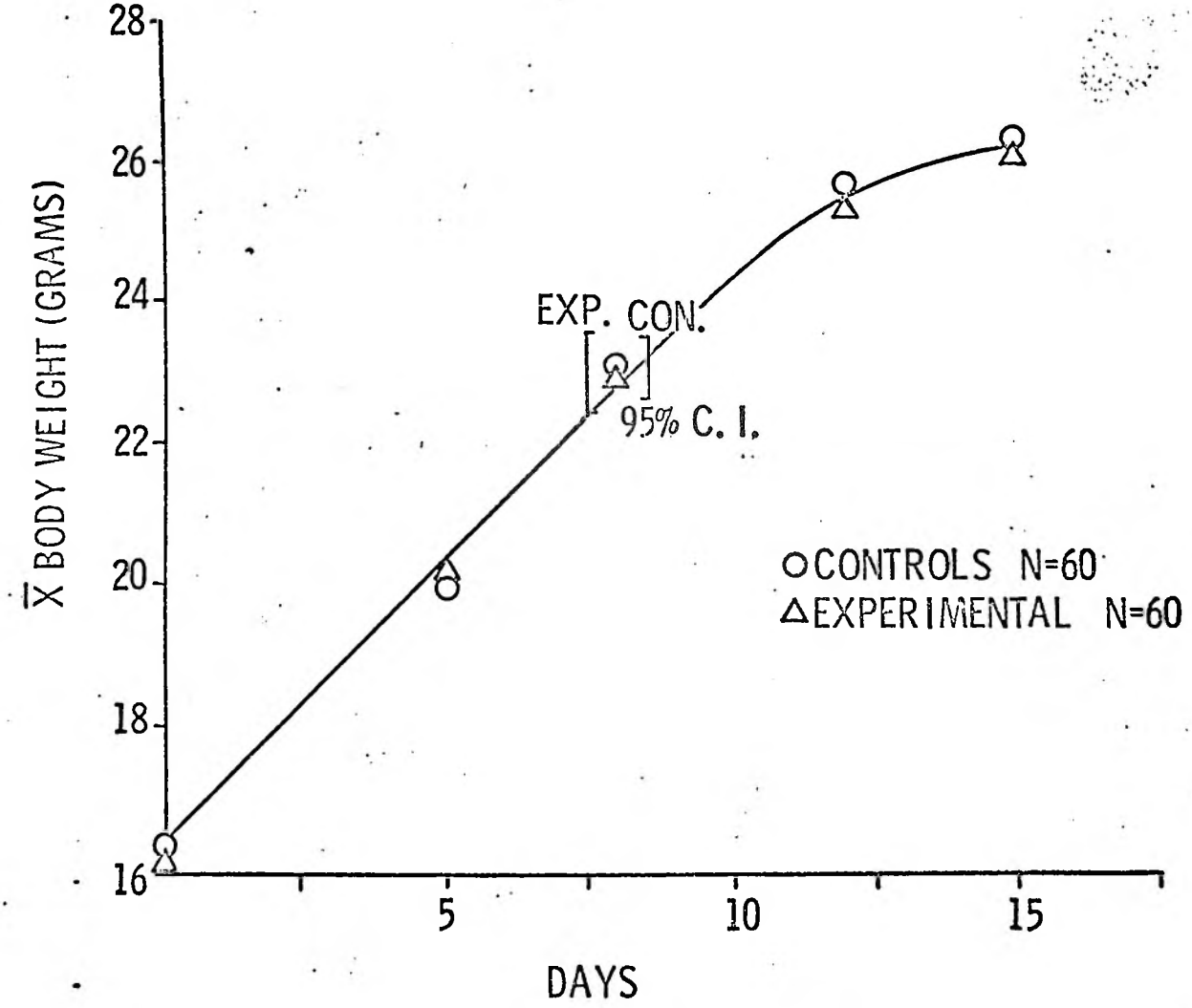
15
20
25
DAYS

Figure 4.

Growth curves of control and treated mice exposed to ELF environment of 75 Hz at 100 V/m. N = 60 for each group.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$\bar{X}$ BODY WEIGHT (GRAMS)
EXP. CON.
95% C. I.
CONTROLS N=60
EXPERIMENTAL N=60
5
10
15
DAYS

Figure 5.

Cumulative mortality rates of mice challenged intranasally with a standard dose of influenza virus. 20 controls and 20 mice exposed. to ELF environment of 75 Hz at 100 V/m. for 21 days.

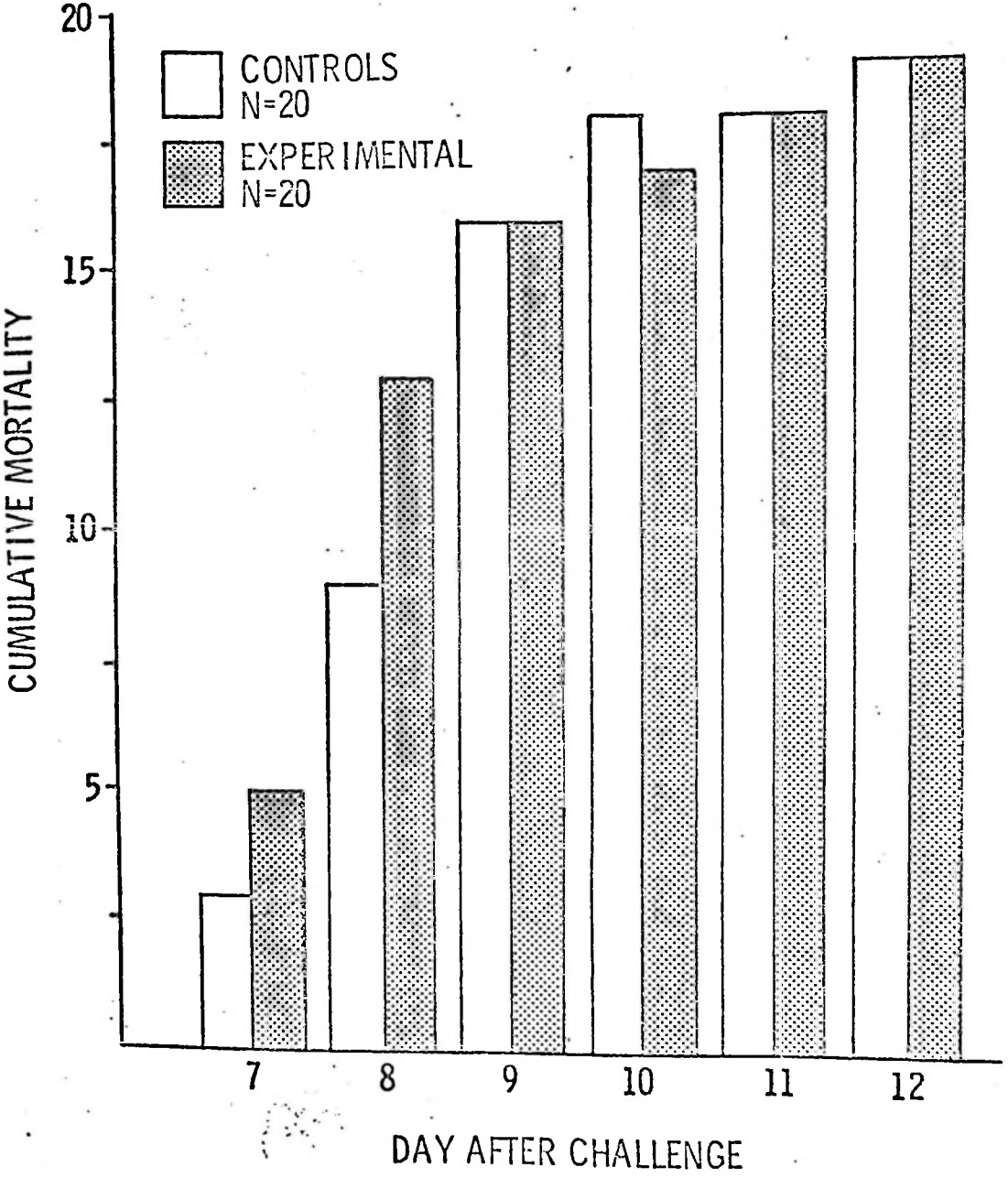
20
15
10
5
CONTROLS
N=20
EXPERIMENTAL
N=20
CUMULATIVE MORTALITY
7
8
9
10
11
12
DAY AFTER CHALLENGE

TABLE 1. Blood and brain serotonin levels of control and treated mice exposed to ELF environment of 45 Hz at 100 V/m. Blood samples were taken at 30 days and brains were removed at 37 days. N=60 for controls and 59 for experimental group.

		$\bar{X}$	95% Confidence Interval	Upper Limit	Lower Limit
Blood 5 Ht μg/ml	Controls	7.293	.499	7.792	6.794
	Experimental	7.541	.467	8.008	7.074
Blood 5 Ht μg/gm	Controls	.811	.054	.871	.763
	Experimental	.773	.058	.821	.715

TABLE 2. Blood and brain serotonin levels of control and treated mice exposed to ELF environment of 75 Hz at 100 V/m. Blood samples were taken at 28 days and brains were removed at 31 days. N=60 for each group.

		$\bar{X}$	95% Confidence Interval	Upper Limit	Lower Limit
Blood 5 Ht g/ml	Controls	6.922	.553	7.475	6.369
	Experimental	7.548	.806	8.354	6.742
Blood 5Ht g/gm	Controls	1.003	.071	1.074	.932
	Experimental	.998	.083	1.081	.915

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BIBLIOGRAPHY

1. Krueger, A.P. (1970): The effect of air ions on bacterial and viral pneumonia in mice. Int. J. Biometeor., 14:247-260.

2. Krueger, A. P., Andriese, P. C., and Kotaka, S. (1963): The biological mechanism of air ion action: The effect of CO_2^+ in inhaled air on the blood level of 5-hydroxytryptamine in mice. Int. J. Biometeor., 7:3-16.

3. Krueger, A. P., et al. (1971): The course of experimental influenza in mice maintained in high concentrations of small negative air ions. Int. J. Biometeor., 15(1):

4. Krueger, A. P., et al. (1966): The effects of inhaling non-ionized or positively ionized air containing 2-4% CO_2 on the blood levels of 5-hydroxytryptamine in mice. Int. J. Biometeor., 10:17-28.

5. Krueger, A.P., et al. (1974): Further observations on the effect of air ions on influenza in the mouse. Int. J. Biometeor., 18(1): 46-56.

6. Krueger, A. P., et al. (1968): Small air ions: Their effect on blood levels of serotonin in terms of modern physical theory. Int. J. Biometeor., 12:225-239.

7. Krueger, A. P., et al. (1962): Studies on the effects of gaseous ions on plant growth. II. The construction and operation of an air purification unit for use in studies on the biological effects of gaseous ions. J. Gen. Physiol., 45:897-904.

8. Krueger, A. P. and Kotaka, S. (1969): The effect of air ions on the brain level of serotonin in mice. Int. J. Biometeor., 13:61-73.

9. Krueger, A. P. and Reed, E. J. (1972): Effect of the air ion environment on influenza in the mouse. Int. J. Biometeor., 16(3): 209-232.

10. Krueger, A. P. and Reed, E. J. (1974): The influence of air ions on a model of respiratory disease. Presented at the First World Congress of Medicine and Biology of the Environment. Paris, France, July 1-5.

11. Reed, L. J. and Muench, H. (1938): A simple method of estimating 50% end points. Amer. J. Hyg., 27:493-497.

12. Reiter, R. (1972): Introductory remarks, Symposium on biological effects of natural electric, magnetic and electromagnetic fields. Sixth International Biometeorological Congress, September 1972. Int. J. Biometeor., 17:205-206.

13. Sulman, F. G., Levy, D., Levy, A., Pfeifer, Y., Superstine, E., and Tal, E. (1974): Int. J. Biometeor., 18(1):313-318. Air-Ionometry of Hot, Dry Desert Winds (Sharav) and Treatment with Air Ions of Weather-Sensitive Subjects. Int. J. Biometeor., 18(1):313-318.

14. Tromp, S. W. (1968): A survey of recent advances in electro-biometeorology. Reports of the Biometeorological Centre X, 22 June 1968.

15. Udenfriend, S., Weissbach, H. and Clark, C. T. (1955): The estimation of 5-hydroxytryptamine (serotonin) in biological tissues. J. Biol. Chem., 215:337-343.

16. Worden, J. L. (1953): The effect of unipolar ionized air on the relative weights of selected organs of the golden hamster. Science Studies, St. Bonaventure University, 15:71.