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The Effects of Long-Term Air Ion and D.C. Electric Field Exposures on Survival Characteristics in Female NAMRU Mice[1]

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Two hundred female NAMRU mice (25 per cage) underwent chronic exposures to the following conditions: positive or negative air ions (2×10^5/cm^3 and 2×10^3/cm^3), D.C. fields only (2 kV/meter), and two identical electrically grounded cages. Survival data yielded median survival times (MSTs) with similar environments usually having equivalent MSTs. Field cages had the longest (661 days) and the negative ion cages the shortest (585.9 days) MSTs. Pairwise comparisons of survival characteristics using Lee-Desu statistics revealed significant differences between environments, with combined ionized compared to nonionized conditions having the most significance ($p < .013$). Mice in general showed a substantial (42%) and significant decrease in serum glucose values with age, with ionized mice having consistently lower glucose levels than nonionized ($p < 10^{-6}$) over the entire exposure period. These results with air ions and D.C. fields argue for the involvement of bioelectrical processes in mortality and aging rate.

Key Words: Bioelectricity, Electromagnetic effects, Life span, Aging

SCIENTISTS have studied the occurrence and possible effects of atmospheric electricity since the discoveries of Benjamin Franklin in 1752. Shortly thereafter, other investigators postulated that atmospheric electricity in nature stimulates plant growth and also might have effects on human health and disease. Studies of the natural environment have indicated bipolar levels of small air ions of up to 10^4/cm^3, with a ratio of about 1:1 between positive and negative species except under special circumstances. Since the 1950s, numerous papers have reported on the biological effects of air ions, positively and negatively charged molecular clusters found in the atmosphere of varying size, mobility, and chemical composition (Kellogg, 1984).

Well-documented effects of experimentally produced air ions (usually of one polarity) include the killing of bacteria, accelerated growth and development in plants and insects, and physiological and behavioral changes in animals and man (see reviews by Kellogg, 1984; Krueger, 1982; Krueger & Reed, 1976). Because of the probable mechanism of the effects of air ions, whereby they would affect endogenous direct current control systems (Kellogg, 1984), these studies belong properly to the emerging discipline of bioelectricity. Becker and Marino (1982) recently reviewed an impressive body of evidence demonstrating the importance of endogenous bioelectric phenomena in such processes as morphogenesis, growth, and regeneration. Numerous studies in the gerontological literature, such as that by McCay et al. (1939), Goodrick (1978), and more recently Cheney et al. (1983), have demonstrated an interdependence between growth and aging processes. Thus, one can speculate that endogenous bioelectrical control systems may play an important role in the regulation of aging processes and that anything that would seriously perturb such controls would have an effect on aging itself.

As an extension of a long-term study evaluating the effects of chronic exposure to monopolar air ions in female NAMRU mice (Kellogg et al., 1985 a, 1985 b), we decided to look for possible aging effects in this experimental group. As monopolar air ions seem as a general rule to increase growth processes, we hypothesized that long-term exposures might augment the aging rate. Our findings on the effects of long-term, continuous exposures of female NAMRU mice to defined air ion and D.C. field environments on lifespan and survival characteristics forms the basis of this report.

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MATERIALS AND METHODS

Exposure environments. — We designed the cages used in this study to provide environments having far more defined air ion and field conditions than have prevailed in past research. Cages provided D.C. field and ion environments with substantial shielding from A.C. noise, as well as a waste disposal system that removed both ammonia and other volatile pollutants (Figure 1). The shielded boxes and stabilized fields formed an important part of the ion exposure system, providing control of ion distribution and of the electrical environment. Typical electric field measurements (using a Trek model 485 fieldmeter) in one cage for field only conditions gave values at the cage floor of 1.80 ± 0.64 (M ± SD) with the field increasing under high ion conditions to 2.40 ± 0.71 kV/m. During exposures animals remained in nearly constant contact with the electrically grounded cage floor and, as a result, accumulated little static charge.

A tritium foil system provided the air ion source and allowed variation of ion production while holding field gradients fairly constant. Specially selected paper filters placed over the foils in low ion cages reduced ion densities by a factor of 100. Low ion cages typically had an overall average exposure of about 2 × 10^3 ions/cm^3, as compared with high ion cages of 2 × 10^5 ions/cm^3. Detailed mapping of air ion levels using a 2 cm^2 probe connected to a Cary Femtoammeter revealed a regular pattern of local ion exposures on the cage floor, with higher concentrations found in the middle of the cage and the corners. Experiments in high ion cages showed average whole body currents of about 10^{-10} A for an isolated, anesthetized animal. Because of the typical piling up of the animals in the corners during the daytime and variations in animal activity, however, one must expect some independence of individual animal exposure to ions as compared with local ion densities. Nonionized (field and ground) cages had very low air ion levels (less than 100 ions/cm^3), and we have alternatively designated these environments as ion-depleted cages.

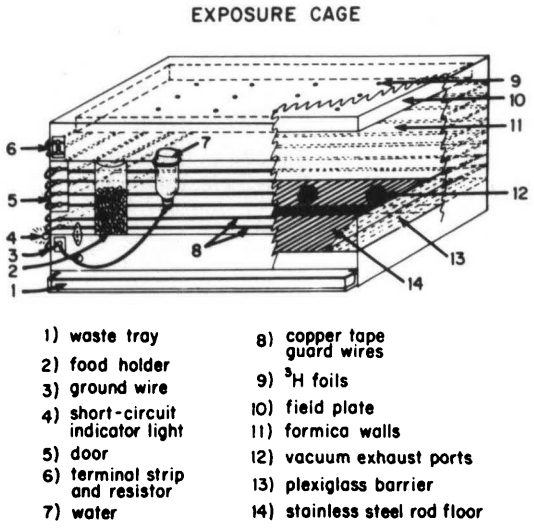

Figure 1. The exposure cage used for air ion research with the right corner cut away to show the cage interior. The cage has the external dimensions of 95 cm wide by 45 cm high by 35 cm deep. Ions of either polarity originate from tritium foils located on the field plate mounted inside the top of the cage. Copper tape guard wires (attached to an external resistor network) imbedded in the cage walls stabilize the electric field between the field plate and the grounded expanded metal floor where animals live. Mice obtain food and water from containers mounted on the cage door. The design allows control of ion density with only small changes in the bias potential between the field plate and the floor.

A total of eight cages resided in two adjacent cubicles, at four different shelf heights. Cage conditions included high negative (HN) and high positive (HP) ions, low negative (LN) and low positive (LP) ions, negative field (NF) and positive field (PF) only (ca 2.0 kV/m, ion-depleted), and two identical electrically grounded (G_1 and G_2, ion-depleted) cages. Air entering the cubicles 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. We maintained temperature at 20 ° ± 1 °C and monitored but did not control relative humidity, which averaged 40 to 50%. The animals had a light schedule of 12 hr on/12 hr off (Vita-Light full spectrum fluorescent bulbs), with the on cycle beginning at 7 a.m., Pacific Standard Time.

We put considerable effort into neutralizing possible variations of conditions (e.g., temperature, vibration, light exposure, etc.) between cages. As a final precaution, we set up a weekly animal rotation schedule, which moved animals from cubicle to cubicle, shelf level to shelf level, and cage to cage, with the cages reset to the appropriate conditions. We did *not* clean cages before rotations, so that we had little if any isolation of the animals in different cages from infections or disease. Similarly, animals from all cages used the same holding cages and bleeding restraints. This insured uniform exposures to pathogens for all animals and eliminated the chance of a variability in disease exposure

having an artifactual effect on results on animals in different cages.

Mice. — Two hundred female mice of the NAMRU outbred strain (born between the 15th and 17th of December, 1981) delivered specific pathogen free (from the Naval Biosciences Laboratory NSC, Oakland, CA 94625) began exposure in our cages (25 per cage) at 6 weeks of age on January 26, 1982. The NAMRU strain, line bred to minimize inbreeding, has a strictly hybrid origin derived from the ABC (Albino-Black-Cross) strain in 1943. (A short pamphlet entitled "History of the NAMRU mouse" available on request from the Naval Biosciences Laboratory.) We picked the NAMRU strain of mice because previous work had shown that these mice respond significantly in a variety of ways to ionized environments (Krueger, 1982). We chose female mice for this study because earlier work done in this laboratory had found male mice too aggressive, with an unacceptable incidence of fighting and injury when maintained in the cages used here in long-term exposure experiments.

Sampling periods occurred every 3 months and consisted of two separate tail-bleeding weeks (from 9 to 10:30 a.m., Monday through Friday, with the cage order varied daily) for blood chemistry assays, with a 2-week recuperation period between bleeding weeks. We used Sigma kit No. 115A to determine serum glucose (not fasted) levels. All mice had their body weight taken monthly from exposure onset. Comparison of nonionized versus ionized animal populations indicated no difference between groups, with body weights differing by an average of only 1.1 ± 1.9% for all monthly weighings. Animals had nearly continuous exposure to the cage environments, except for about 6 hr every 2 weeks when the cages underwent a thorough cleaning procedure.

Animals ate autoclavable rodent laboratory chow (Purina 5010) as their standard diet. In early September of 1982 we noticed that some of the animals had extremely raw and irritated snouts, which resulted primarily from the animals' roughening of the surface of their plexiglass feeders. We believe that a mild vitamin deficiency, resulting from food overautoclaved in the animal services department, exacerbated this problem. We removed the affected animals (about two per cage average) and after trying a variety of treatments, decided to exclude them from the study. We also firepolished and smoothed the plexiglass feeders and decided from this point on to autoclave the food ourselves (15 min in small batches), which prevented any further incidence of this problem. One should bear in mind, however, that all of the experimental animals probably suffered from a mild vitamin deficiency as a background stress from the beginning of our study until October of 1982.

Survival Data and Statistical Analysis

Analysis of individual survival dates recorded for the 200 animals in our study used the Statistical Package for the Social Sciences (SPSS) survival procedure (Hull & Nie, 1981). To make full use of the available data, we included accidental deaths and animals withdrawn from the study as censored observations and calculated survival functions (cumulative survival, probability density, and hazard rate) using product-limit estimates (Kaplan & Meier, 1958). Life tables based on daily intervals for each of the eight cages yielded exact median survival times and survival functions for different exposure conditions. We compared the survival experience of different exposure groups using the Lee-Desu D statistic available in SPSS (Lee & Desu, 1972). A pairwise comparison of the two ground condition cages showed no significant difference between these cages $D(1) = .20, n = 50, p < .658$, so subsequent analysis used pooled data from these two cages, resulting in seven unique exposure groups.

An overall comparison showed a significant difference $D(6) = 12.6, n = 200, p < .05$, for the cumulative survival of the seven different exposure groups. Further pairwise comparisons using SPSS determined which groups contributed to this significant result. Many of the significant differences occurred between ion-exposed and nonexposed cages, whereas cages with similar ionization conditions such as HN and LN showed no significant differences. We aggregated the data into two environmentally similar groups, each containing 100 animals, representing ion-exposed (HN + LN + HP + LP) and nonion-exposed conditions (NF + PF + G_1 + G_2). We also compared the survival experience of these two groups using the D statistic from SPSS.

Plots of the cumulative percent survival for the various groups derived from the data of the life table calculations. These plots used 14-day interval data rather than single day figures, and the Statistical Analysis System (SAS) gplot procedure to fit smooth cubic spline curves to the survival data from the life tables.

To assess differences in serum glucose levels between ionized and nonionized conditions over

the entire exposure period we used the SPSS analysis of variance (ANOVA) procedure with both exposure condition and time designated as independent variables. The Multiple Classification Analysis (MCA) available in the SPSS ANOVA procedure compared the average response due to exposure (adjusted for time effects) with the grand mean for all the data and computed probability values for these comparisons using F rations. This gave a measure of overall differences in biochemical response for ionized versus nonionized exposure groups, controlling for differences occurring over time.

PATHOLOGY

During the latter half of this study we checked cages at least every other day for animal deaths. Dead mice in reasonable condition (not overly cannibalized or decomposed) received autopsies, with unusual tissue or organ samples saved for later analysis. On the termination dates of this study (November 2 and 3, 1983) we performed more complete autopsies on the surviving animals, taking organ weights, and saving unusual samples for histological analysis. Overall, we found no gross differences in animal pathology or disease incidence between ionized and nonionized conditions. Some animals from all cage conditions showed evidence of Proteus infections beginning sometime during the first year of exposure. Many of the animals had severe gastroenteritis, or infections of the fallopian tubes, and displayed hypertrophy of the spleen and hepatitis at death. With the exception of tests used to confirm the nature of the Proteus infection, we performed no other tests for pathogen contamination during the course of the experiment. Although the Naval Biosciences Laboratory maintains the NAMRU strain "specific pathogen free" under barrier conditions, we made no effort to maintain this condition over the course of the long-term exposures. Occasionally, we also observed nephrosis of the kidney and lung adenomas, which occur fairly commonly in the NAMRU mouse strain. Due to the incomplete nature of the pathology data base, we cannot validly compare in any statistical way the incidence of pathologies between different exposure conditions. It did seem clear, however, that no exposure condition had any unique pattern of pathology associated with it.

RESULTS

Mortality. — Analysis of the survival data yielded median survival times (MSTs) for each of the cage conditions, as well as values for combined populations (e.g., all negative ion cages, all ionized cages). Table 1 presents these results. The MSTs show remarkable consistency between similar conditions, with the two field (NF and PF) cages having the longest (661 days) and the negative ion cages (HN and LN) the shortest (585.9) combined MSTs. Pairwise comparisons of survival characteristics using Lee-Desu statistics (Table 2) revealed a number of significant differences between environments. Combined data for both negative ion versus field cages, which differed in median survival time by 12.8%, had a p value difference less than .016. Of even more interest, a comparison of all mice in ionized cages (HN, LN, HP and LP) versus nonionized cages resulted in a significant difference ($p < .013$) for survival characteristics between groups. These statistics argue strongly for an important and adverse effect of nearly lifelong exposures to ionized, as compared with ion-depleted, environments on the survival characteristics of female NAMRU mice.

The equivalence of results between similar or identical cage habitats argues for the reproducibility of the cage environments and qualitative ion effects in the compared cages. Pairwise compari-

Table 1. Cage Environment Median Survival Times

Cage conditions	Median survival time (days)	Cage conditions	Median survival time (days)
High negative ions (HN)	575.5	Negative field	674.5
Low negative ions (LN)	610.7	Positive field	656.5
All negative ions (NI)	585.9	All field cages	661.0
High positive ions (HP)	662.7	Ground 1	631.0
Low positive ions (LP)	599.5	Ground 2	622.0
All positive ions (PI)	620.2	All ground cages	630.0
All ion cages	610.2	All non-ion cages	651.0
All cages	624.8		

sons of the negatively ionized cages (HN vs. LN, $D(1) = .05$, $n = 50$, $p < .832$), the two ground cages ($p < .658$) and the two field cages (NF vs. PF, $D(1) = .04$, $n = 50$, $p < .842$) yielded results close to unity, indicating marked similarity of the survival characteristics between paired cages. A comparison of field versus ground cages (all nonionized $D(1) = .62$, $n = 100$) yielded a p value less than .430.

In marked contrast to these results, however, the two positively ionized cages (HP and LP) did have statistically significant differences in survival characteristics ($p < .028$), which may indicate different effects for positive air ions at these two levels of exposure.

Figure 2 presents the combined survival curves for mice in the ionized (HN, LN, HP and LP) compared with ion-depleted (NF, PF, G_1 and G_2) cages. Although we have no data on the maximal lifespan of these animals (because of the experimental termination date required by the terms of our contract) the curves approach a rectangular form consistent with fairly optimal environmental conditions. The two curves depict the difference between ionized and ion-depleted cage conditions. Unfortunately, we have not found any study in the literature describing life span characteristics of NAMRU mice under more standard conditions, so we cannot compare our survival curves with others for this strain.

Table 2. Pairwise Comparisons of Survival Characteristics Using Lee-Desu Statistics (all pairs with $p < .05$)

Compared conditions	$D(1)$[a]	n	p
High negative ions vs. positive field	4.61	50	.032
High positive ions vs. low positive ions	4.80	50	.028
Low positive ions vs. negative field	4.93	50	.027
Low positive ions vs. positive field	6.02	50	.014
Low positive ions vs. ground	4.36	75	.037
Negative ions vs. field	5.78	100	.016
Negative ions vs. ground	4.05	100	.044
Ionized vs. nonionized	6.16	200	.013

[a]The chi-square has 1 degree of freedom.

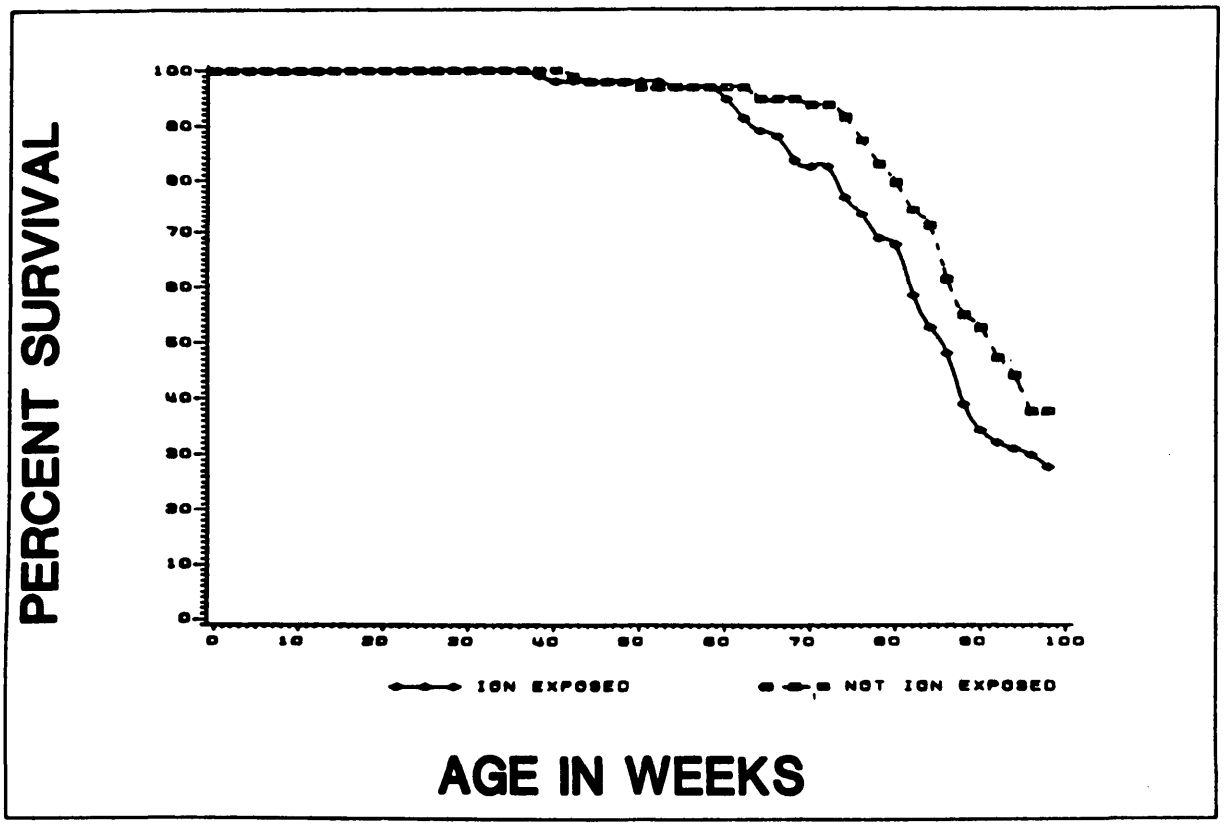

Figure 2. Survival curves for female NAMRU mice maintained in ionized (HN + LN + HP + LP) or nonionized (NF + PF + G_1 + G_2) environments.

Glucose. — On the basis of the survival differences found between ionized and nonionized cages, we reanalyzed previously collected data for serum chemistries for the 2 years of this study (Kellogg et al., 1985a, 1985b). This resulted in the finding that animals in ionized cages had consistently lower serum glucose levels when compared with nonionized cages (Figure 3). Multiple Classification Analysis of serum glucose for ionized versus nonionized environments yielded a significant p value less than 10^{-6}, F (1, 1257) = 31.1. Serum glucose also exhibited a substantial (42%) and significant F (7, 1257) = 152, $p < 10^{-6}$, decrease with age. In this respect, it seems interesting to note that ionized cages, which had a shorter median survival time than did the nonionized cages of about 6 weeks, displayed serum glucose levels approximately corresponding to those seen for animals in nonionized cages 10 weeks older in age. This suggests the possibility of accelerated aging process seen for the ionized, as compared with the nonionized group.

Discussion

In the past 25 years the Air Ion Laboratory has studied the response of a variety of living forms to air ions in controlled environments. These studies have investigated effects on bacteria, higher plants, and small animals. Small air ions of both polarities have caused greatly enhanced growth and increased yield in a variety of plant studies (Krueger et al., 1962; Pohl & Todd, 1981; Yamaguchi & Krueger, 1983), using different ionization sources, plant species, and performed in many different laboratories. Another well-controlled study by Krueger et al. (1966) demonstrated marked acceleration of growth and maturation, using a number of different parameters, in silkworms exposed to positive or negative air ions. More recently, Diamond et al. (1980) reported on a very interesting study using Long-Evans rats, where they show that animals exposed to negative ions from a corona discharge source for 3 months (in multifamily enriched conditions) had significant increases in cortical wet weight in both the somatosensory and occipital areas of the brain as compared with control animals in nonionized cages. Although our long-term exposure study showed insignificant differences in body weight or growth between nonionized and ionized groups (1.1% ± 1.9 *SD* average for the 24 monthly weighings), this finding does not rule out the possibility of differences in more subtle processes of growth such as seen in Diamond et al.'s study. As a general rule continuous exposure to monopolar air ions accelerates growth processes that could thereby accelerate the aging rate. Studies by McCay (1939), Goodrick (1978), and Cheney et al. (1983) support the hypothesis of an interdependence of growth and aging processes.

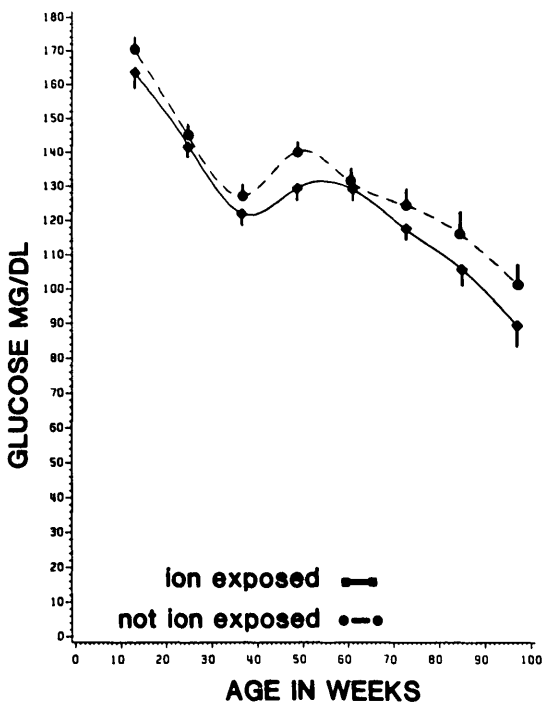

Figure 3. Average serum glucose levels (not fasted) for female NAMRU mice maintained in ionized or nonionized environments.

Our results showed a difference in life span between animals maintained in ionized as compared with nonionized cages, with the ionized cages having significantly shorter life spans ($p < .013$). A Multiple Classification Analysis of serum glucose levels between these two groups, over the entire two year exposure period, yielded a difference with an extremely high significance level ($p < 10^{-6}$), with the ionized cages having consistently lower glucose levels for all sample periods. Of interest also, we found in these female NAMRU mice a continuous and highly significant ($p < 10^{-6}$) downward trend in serum glucose levels from the age of 49 weeks on with a close correlation between the age of the animals with serum glucose levels. Thus, the lower glucose levels seen for ion-exposed animals appears consistent both quantitatively and qualitatively with the possibility of increased physiological age as compared with animals maintained in cages without ions.

Of the various theories propounded to explain air ion effects, the direct current hypothesis holds the most promise in explaining how air ions might

induce the changes seen in the present study (Kellogg, 1984). Becker and Marino (1982) have reviewed the evidence for the roles of an endogenous D.C. control system in biological organisms, and experiments on rat limb regeneration (Becker, 1972) have shown that minute electrical currents with a window at about 5 nA play an essential role in processes of growth and regeneration. Monopolar air ions by their nature cause D.C. electrical currents to flow in grounded subjects along the paths of least resistance, and as it appears that endogenous direct currents play a significant role in maintaining homeostasis, any alteration of that system could have profound physiological effects. The window effect, commonly observed in bioelectrical phenomena, may help explain the discrepancy seen in survival in the two groups subject to positive ion exposure, where the HP group survived significantly longer ($p < .028$) than the LP group. Thus, the evidence reported here on the effects of air ions on survival characteristics offers at least tentative support for the hypothesis that bioelectrical processes play a significant role in aging.

Finally, it seems important to point out that we had as our primary purpose the hope that this work would help "break new ground" in the field of gerontology by introducing data that implicates bioelectrical phenomena in the aging process. At this point we cannot pretend to have proved such involvement and cannot offer other than hypothetical mechanisms for the effects obtained, because work on the mechanisms of even well-validated bioelectrical phenomena has really just begun. The fact remains, that in this study air ion exposures did affect survival characteristics, and these data support the hypothesis that bioelectrical processes can play a role in aging. Up to the present time the possible role of bioelectric processes in aging has remained almost entirely unexplored. We hope that future studies will investigate more thoroughly the possibilities of this new approach to the aging process.

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