The Effect of Continuous Exposure to Low Frequency Electric Fields on Three Generations of Mice: A Pilot Study[1]

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Summary. Mice were allowed to mate, gestate, deliver and rear their offspring for 3 successive generations while being continuously exposed to 60 Hz electric fields. Mice exposed to vertical electric fields exhibited decreased body weights at 35 days postpartum and increased mortality rates for 3 successive generations. Mice exposed to horizontal electric fields exhibited decreased body weights for 2 successive generations.

Recent reports have described a variety of subtle biological effects due to exposure to low frequency electric fields. Electric fields of 10 Hz have been shown to affect human circadian rhythms[3], and to alter skin potential in frogs[4]. Electric fields of 50 Hz have been found to produce cardiovascular effects in mice and rats[5], and to affect the mitotic index of liver cells in the mouse[6]. Electric fields of 60 Hz have induced bipolarity in *Dugesia*[7], and have been shown to be detectable by fish[8]. No studies have been done to assess the possible impact on successive generations of animals from the continuous presence of a low frequency electric field. This report describes such a study.

Initially, mature male and female Ha/ICR mice were purchased commercially and split into horizontal, vertical, and control groups. Mice in the horizontal group were allowed to mate, gestate, deliver, and rear their offspring in a horizontal 60 Hz electric field of 100 volts/cm. At maturity, randomly selected individuals from the 1st generation were similarly allowed to mate, gestate, deliver, and rear their offspring while being continuously exposed. Randomly selected individuals from the 2nd generation were then mated to produce the 3rd and final generation. A parallel procedure was followed for the vertical group wherein 3 generations were produced in a 60 Hz vertical electric field of 150 volts/cm, and for the control group wherein 3 generations were produced in the ambient electric field.

Breeding was accomplished by allowing 2 females and 1 male to occupy a single cage. Pregnancy was determined by abdominal palpation. Pregnant females were placed in individual cages and remained with their offspring until weaning, at about 3 weeks after birth. The number of offspring in each litter was determined daily, beginning on the day of birth. After weaning, the mice were separated by sex and their body weights were measured periodically up to 35 days after birth, except in the case of the 2nd generation which was weighed up to 10 weeks postpartum.

During the study, all mice were housed in a room having a temperature of 23 °C, a relative humidity of 50% and a light-dark cycle of 12:12. Plastic cages (15 × 30 × 15 cm) with metal cage tops were employed, except for the horizontal group which had plastic cage tops. The mice had continuous access to water via a water bottle with a metal straw which protruded about 5 cm downward from

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[2] Vibration measurements were performed by Dr. Daniel A. Driscoll, New York State Department of Environmental Conservation.

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Effect of low frequency electric fields on three successive generations of mice

Treatment	A	B	C	D	E	F	Average wt. (g) 35 days after birth		Average wt. (g) 10 weeks after birth	
							Female	Male	Female	Male
1st Generation										
Vertical electric field	8	5	52	10.4	4%	10%	(23) 17.0[a] ± 3.2	(22)22.8[a] ± 4.9	—	—
Horizontal electric field	8	5	47	9.4	0	2%	(21) 22.0[a] ± 1.8	(25) 23.9[a] ± 3.4	—	—
Control	8	5	61	12.2	15%	2%	(26) 24.0 ± 1.5	(25) 27.0 ± 2.0	—	—
2nd Generation										
Vertical electric field	8	7	81	11.5	0	58%	(17) 13.6[a] ± 2.8	(15) 15.4[a] ± 2.9	(16) 27.5[b] ± 2.5	(15) 29.2[a] ± 2.6
Horizontal electric field	8	8	92	11.5	3%	0	(43) 19.2[a] ± 2.7	(47) 22.8[a] ± 2.8	(28) 27.2[a] ± 2.3	(39) 33.7[a] ± 3.0
Control	8	8	91	11.4	1%	4%	(42) 23.6 ± 1.7	(44) 27.5 ± 2.8	(34) 29.9 ± 2.5	(32) 36.5 ± 2.9
3rd Generation										
Vertical electric field	8	6	54	9.0	0	35%	(19) 17.6 ± 3.8	(16) 17.3[a] ± 4.7	—	—
Horizontal electric field	8	8	102	12.8	14%	3%	(42) 20.9 ± 2.5	(43) 23.3 ± 2.9	—	—
Control	8	8	98	12.2	0	2%	(47) 19.2 ± 3.3	(49) 21.7 ± 3.8	—	—

A, number of females mated; B, number of pregnancies; C, number of pups delivered; D, average litter size; E, mortality during 1st week postpartum; F, mortality during 8–35 days postpartum. The number of mice is given in parenthesis. [a] $p < 0.001$. [b] $p < 0.01$.

the cage top. Continuous access to food was provided via a trough which protruded downward from the cage top about 7 cm. In each case, the trough was constructed of the same material as the cage top.

The vertical electric field was generated by grounding the metal cage top and applying the appropriate voltage to an insulated metal plate which was placed under the plastic cage. The horizontal electric field was generated by employing a suitably mounted capacitor in which neither the energized plate nor the grounded plate made physical contact with the plastic cage. The relatively high strength vertical and horizontal electric fields employed resulted in electric field induded vibration in the vicinity of the cages of about 2.5×10^{-8} cm/sec, which was smaller than the ambient vibration in the absence of the electric fields.

The results are given in the Table. In the 1st generation, males and females reared in both the horizontal and vertical electric field were significantly smaller than the controls when measured at 35 days postpartum. Larger depressions in average body weight were seen in the 2nd generation at 35 days postpartum, while at 10 weeks postpartum the differences between the experimental and control weights had narrowed considerably. A very large mortality rate in the vertical field mice during the 8-35 day postpartum period was also noted. A large mortality rate was again seen in the vertical groups in the 3rd generation, however the only group whose body weights were significantly affected were the males exposed to the vertical electric field.

The mice exposed to the electric fields demonstrated obvious effects compared to the equivalent control mice. The most severe effects were seen in the males and females exposed to the vertical field, possibly due to the greater intensity of the vertical field. Alternatively, a direction-dose factor may be involved. In the vertical field experiments, a relatively constant dorsi-ventral exposure vector existed, particularly for the central nervous system, regardless of the movement of the mice. In the horizontal field, the relationship between the mice and the field direction was constantly changing as a result of their movement. The increased severity in the vertically exposed mice may therefore indicate the existence of a directionally sensitive sensing mechanism within the mouse which initiates a response proportional to the time the electric field is along a certain axis.

The vertically exposed mice experienced (after weaning) microcurrents of the order of 5 μA when eating or drinking, because both acts necessitated touching grounded conductors. The horizontally exposed mice experienced much less microcurrent because their entire cage was constructed of plastic. The possibility must therefore be considered that the greater weight depressions and the increased mortality in the vertical mice may be related to the grounding microcurrents.

Long term exposure to altered environmental conditions may lead to adaption via a variety of mechanisms including exclusion of susceptable individuals from the genetic pool by death prior to maturity or by favoring the survival of those genetically constituted to better resist the altered circumstances. The elevated 8–35 days mortality rate in the 2nd generation, and the decreased severity of the weight differentials between the experimental and control mice in the 3rd generation may be interpreted as evidence for such a mechanism. On the other hand, the elevation of the 8-35 day mortality rate in the 3rd generation is some evidence to the contrary. More extensive studies are necessary to explore this possibility, as well as to explore the basic causative factors for the effects described herein.

Cytokinin Contents and cAMP Metabolism During Growth of *Escherichia coli*[1]

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Summary. During *Escherichia coli* growth, we found an inverse correlation between free cytokinin content and cAMP level. The rates of synthesis of adenylate-cyclase and cAMP-phosphodiesterase were practically constant.

At present our knowledge of the role of cytokinins (N^6-derivatives of adenine) in microbial physiology is still scarce[3–5]. Recent evidence has shown, for a wide range of biologically active compounds, including cytokinins[6–9], an action correlated to the adenosine, 3′:5′ monophosphate (cAMP) system[10]. Since cytokinins do not induce as evident effects on microorganisms as on animals and higher plants[11], in order to verify a possible interaction between cytokinins and cAMP in microbial metabolism, as a first approach, we measured cytokinin and cAMP levels together with the rate of synthesis of the enzymes responsible for cAMP metabolism during a cultural cycle of *Escherichia coli*.

Materials and methods. Escherichia coli, B/b strain, kindly provided by Dr. M. L. BARNETT, Cambridge University, England, was used. Bacteria were aerobically grown in M9 Salts Medium[12] at 37 °C. Growth was measured turbidimetrically and by direct counts in a Petroff-Houser chamber. Intra- and eso-cellular cAMP was measured according to BUETTNER et al.[13], using the protein-binding assay of GILMAN[14]. Presentation of intracellular concentrations of cAMP in units of molarity is based on an accessible volume of 4.7×10^{-12} ml/bacterium during the first 120 min of growth, and on a volume of 4.28×10^{-12} ml/bacterium for the residual time. At 30 min intervals, 5 l of culture were rapidly cooled to 1 °C and centrifuged by a continuous MSE H.S.18 apparatus. The extraction of free cytokinins from known weights of wet cells was carried out according to EINSET and SKOOG[15]. The cytokinin activity of the diluted extracts was measured according to VAN ONCKELEN and VERBEEK[16]. For the enzyme assays, washed cells were added to glass beads, 2 parts in weight, and 60 m*M* pH = 7.5 *Tris*-HCl buffer, 3 parts in volume, and disrupted in a Braun Supercell-homogeneizer. The homogenate was centrifuged for 30 minutes at $30{,}000 \times g$, and the fluid supernatant was directly employed in enzyme reactions. The same extract was used in blank reactions after a 3 min treatment in boiling water. Adenylate-cyclase assays were carried out according to BÜRK[17], by recording ^{14}C-cAMP increases. In the cAMP-phosphodiesterase assays the reaction mixture contained, in 60 m*M* pH = 7.5 *Tris*-HCl, 0.5 m*M* ^{3}H-cAMP, 2 m*M* $MgCl_2$, 2.5 m*M* dithioerytritol; ^{3}H-cAMP decreases were controlled.