Sensitivity to change in electrical environment: a new bioelectric effect

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MARINO, ANDREW A., JAMES M. CULLEN, MARIA REICHMANIS, ROBERT O. BECKER, AND FRANCIS X. HART. *Sensitivity to change in electrical environment: a new bioelectric effect.* Am. J. Physiol. 239 (Regulatory Integrative Comp. Physiol. 8): R424–R427, 1980.—The action of a 60-Hz, 5 kV/m electric field on erythrocyte parameters in mice was determined. No effects attributable to the magnitude of the field were found, but a transition either from or to an environment containing the field caused decreased red blood cell concentrations and decreased hematocrits. The failure of others to observe effects on erythrocyte parameters following exposure to low-frequency electric fields may have been due to an inappropriate choice of duration of exposure.

mice; 60-Hz electric field; erythrocytes; stressor

EXTREMELY LOW-FREQUENCY (ELF) electric and magnetic fields—less than 100 Hz—have produced biological effects in subjects ranging from amoeba to man (6). The role of exposure duration is among the many unanswered questions concerning such bioeffects. It might be supposed that longer exposure periods would result in either stronger effects, or increased probability of observing an effect, but often this is not the case. Exposure of human beings to ELF fields for 1 day or less resulted in increased leucocyte concentrations (5), increased triglycerides (2), and altered psychological test performance (4). Also, ELF electric fields of less than 1 kV/m have altered the motility of amoebas in 10 min (3), the calcium efflux from cat cerebral tissue in 20 min (1), and the mitochondral function of guinea pig brain cells in 90 min (11). On the other hand, rats exposed to a 100 kV/m, 60-Hz electric field for 15–20 days exhibited no change in 29 blood parameters (10).

There is, presently, little hope of satisfactorily understanding the ELF-field effects on the basis of fundamental biophysical principles. Two important reasons are that the electrical constants of biological tissue such as the dielectric constant and conductivity are not well characterized, and that it is very difficult to directly measure the fields inside living organisms. These problems have prevented the calculation of unique or verifiable internal field levels. For example, let us model a mouse as a sphere of tissue of radius a, surrounded by a layer of skin of thickness b-a, and subjected to an applied electric field of $E_0\cos\omega t$ with angular frequency ω. Let the conductivities (g), dielectric constants (K), and fields (E) in each of the two regions be denoted, respectively, as follows: g_s, g_t; K_s, K_t; E_s, E_t. It can be shown that

$$E_t = [(1 + D_2/a^3)C_1 + D_1C_2/a^3]\cos\omega t - [(1 + D_2/a^3)C_2 - D_1C_1/a^3]\sin\omega t$$

$$E_s = [(D_2C_1 + D_1C_2)/r^3 - C_1]\cos\omega t + [(D_2C_2 - D_1C_1)/r^3 - C_2]\sin\omega t$$

where

$$C_1 = R_2C_2/R_1 + S/R_1$$

$$C_2 = -SR_2/(R_1^2 + R_2^2)$$

$$S = 3\omega\epsilon_0 b^3E_0/2$$

$$R_1 = g_sD_1 + \omega\epsilon_0D_2(K_s - 1) - \omega\epsilon_0b^3\,(1 + K_s/2)$$

$$R_2 = g_sD_2 - \omega\epsilon_0D_1(K_s - 1) - g_sb^3/2$$

$$D_1 = 3\omega\epsilon_0Z(K_sg_t - K_tg_s)$$

$$D_2 = Z[\omega^2\epsilon_0^2(K_s - K_t)(2K_s + K_t) + (g_s - g_t)(2g_s + g_t)]$$

$$Z = a^3/[\omega^2\epsilon_0^2(2K_s + K_t)^2 + (2g_s + g_t)^2]$$

and where $a < r < b$ and ϵ_0 is the permittivity of free space. As can be seen in Table 1, the calculated fields in the tissue and skin are strongly dependent on the assumed values of the tissue constants—no one set of which can be said to be correct to the exclusion of the others. More realistic geometries introduce even further variations in the calculated fields.

Despite the present inability to uniquely identify the internal fields, Table 1 shows that the range of uncertainty is far below the levels associated with the known mechanisms for ELF-field:tissue interaction—tissue heating and neural stimulation. It is clear, therefore, that there must exist an as yet unidentified mechanism.

We think that, for now, the most fruitful approach to understanding most aspects of ELF bioeffects is at the systemic level. We have suggested that ELF fields are biological stressors and can elicit physiological changes that are part of the classical stress syndrome (7, 12). During a stress response the body adapts to maintain homeostatic conditions and this leads to strongly time-

dependent alterations in peripheral blood composition (12). The exposure duration, therefore, could markedly influence the likelihood of observing an effect. To explore this possibility and to help provide the biological data needed to successfully frame a physical theory of ELF bioeffects we examined the erythrocyte component of the whole blood of mice in connection with 2-day exposures to an ELF electric field.

METHODS

We looked for changes in hematological parameters of Ha/ICR mice due to short-term exposure to a full-body vertical 60-Hz electric field of 5 kV/m.

To ensure maximum statistical sensitivity every mouse was sampled twice, once after exposure to the field for 2 days and once following a 2-day nonexposure period.

TABLE 1. *Calculated values of electric field and power dissipated inside a mouse modeled mathematically as sphere of tissue surrounded by layer of skin*

Tissue Electrical Constants*		Electric Field, V/m × 10^4		Power Dissipated, W/m^3 × 10^9	
Dielectric constant	Conductivity, $\Omega^{-1}\cdot m^{-1}$	Tissue	Skin	Tissue	Skin
80	1	0.707	3.92	5.00	154
80	0.33	2.08	4.49	14.3	66.5
8×10^6	0.2	2.97	4.48	17.6	40.1
8×10^6	0.02	10.1	61.9	20.4	7.66

Calculated values are strongly dependent on assumed values of electrical constants in each region. For simplicity, only 1 set of skin constants has been considered. In all cases, a = 0.12 m and b = 0.15 m. Values in the skin depend on location; listed values pertain to the outer radius (r = b). * Skin electrical constants: 10, 0.1 $\Omega^{-1}\cdot m^{-1}$.

There were four consecutive experiments, two with males and two with females. In each there were two groups: in one, the control period preceded the exposure period (nF → F) and in the other the pattern was reversed (F → nF).

Our exposure facility has been described in detail (8). Briefly, the mice were housed individually in a nonmetallic cage and vibration, light, light-dark cycle, and temperature were controlled. Two assemblies, each consisting of three pairs of shelves, were used; each shelf was a metal plate sandwiched between two layers of wood. In the assembly that housed the nonexposed mice the plates were electrically grounded, and in the second assembly the lower plate in each pair was energized and the upper place grounded. We applied 1,590 V, which produced 5 kV/m in the living space of each mouse in the exposure assembly; the 60-Hz field in the control assembly was essentially zero. Except for the fields, the environment of each mouse was identical in all respects.

On *day 1* of each experiment the mice were divided into two groups and the electric field was applied to one-half the population. On *day 3* the blood parameters were measured in each mouse and immediately thereafter the exposed and nonexposed groups were interchanged. On *day 5* the blood parameters were measured again and the mice were killed.

Blood was collected from the opthalmic vessels by inserting a 25-μl capillary pipette into the orbit medial to the eye. This procedure was performed in less than 20 s and did not produce any signs of trauma.

Measurements were made using a Clay-Adams HA-5 hematology analyzer. The freshly drawn blood was diluted (1:260) in a phosphate-buffered counting fluid containing an anticoagulant. This dilution was used for hemoglobin (Hb) determinations, and a second dilution was

TABLE 2. *Means ± SD of observed changes in hematologic parameters in mice*

Experiment	Condition	RBC		Hct		Hb		MCV		MCH		MCHC	
		1	2	1	2	1	2	1	2	1	2	1	2
A													
Male control	nF → nF (18)	6.91 ±0.55	7.03 ±0.55	40.0 ±3.5	40.8 ±3.4	13.3 ±1.2	12.7 ±1.0*	57.0 ±2.1	57.6 ±1.0	19.3 ±1.5	18.1 ±1.4*	33.3 ±2.7	31.2 ±2.4*
Female control	nF → nF (20)	6.70 ±0.33	6.96 ±0.32*	38.8 ±2.0	40.4 ±1.8*	12.1 ±0.7	11.9 ±0.6	57.5 ±1.1	57.6 ±0.9	18.1 ±1.2	17.2 ±0.9*	31.2 ±1.6	29.6 ±1.5*
B													
Male I	F → nF (16)	6.14 ±0.70	5.85 ±0.69*	35.4 ±4.6	33.6 ±4.3	NM		57.2 ±1.7	57.2 ±0.7	NM		NM	
	nF → F (16)	6.02 ±0.74	5.71 ±0.70*	34.7 ±4.6	33.0 ±4.5			57.2 ±1.5	57.3 ±1.2				
Male II	F → nF (19)	6.99 ±0.40	6.36 ±0.46*	40.7 ±2.2	37.0 ±2.8*	12.2 ±0.5	11.8 ±0.6*	57.9 ±0.8	57.7 ±1.3	17.6 ±1.0	18.6 ±1.2*	30.2 ±1.6	32.0 ±3.2*
	nF → F (19)	6.89 ±0.62	6.44 ±0.54*	40.0 ±3.7	37.2 ±3.4*	12.3 ±0.6	12.0 ±0.7*	57.8 ±1.5	57.4 ±1.1	18.0 ±1.5	18.7 ±1.2*	31.0 ±2.7	32.9 ±1.9*
Female I	F → nF (18)	6.41 ±0.56	6.15 ±0.72*	37.1 ±3.7	35.4 ±4.8*	11.9 ±0.9	11.4 ±0.8*	57.6 ±1.6	56.9 ±1.5	18.6 ±2.0	18.7 ±1.5	32.3 ±3.6	32.7 ±3.2
	nF → F (18)	6.41 ±0.65	6.00 ±0.71*	37.1 ±3.9	34.6 ±5.9*	11.6 ±1.0	11.2 ±0.6*	57.5 ±1.8	57.2 ±2.9	18.2 ±1.7	18.9 ±1.9	31.5 ±3.3	33.0 ±4.3
Female II	F → nF (20)	7.02 ±0.56	6.65 ±0.52*	41.8 ±3.5	39.3 ±3.4*	11.7 ±1.0	12.1 ±0.8	59.4 ±1.4	58.7 ±1.5*	16.8 ±1.8	18.2 ±1.4*	28.1 ±3.1	30.9 ±2.7*
	nF → F (20)	7.04 ±0.54	6.54 ±0.54*	42.4 ±3.4	38.5 ±3.3*	11.5 ±0.8	11.9 ±0.5	59.9 ±1.4	58.5 ±1.2*	16.4 ±1.4	18.2 ±1.4*	27.3 ±2.6	31.0 ±2.9*

A, no change in exposure condition; *B*, change in exposure condition as indicated. NM, not measured. No. of mice in each group is shown in parentheses. Units of each parameter are listed in text. * $P < 0.05$.

TABLE 3. *Percent change in hematologic parameters*

Experiment	Condition	Change, %					
		RBC	Hct	Hb	MCV	MCH	MCHC
A							
Male control	nF → nF	1.7	2.0	−4.5*	1.0	−6.2*	−6.3*
Female control	nF → nF	3.9*	4.1*	−1.7	0.2	−5.0*	−5.1*
B							
Male I	F → nF	−4.7*	−5.1	NM	0	NM	NM
	nF → F	−5.2*	−4.9	NM	0.2	NM	NM
Male II	F → nF	−9.0*	−9.1*	−3.3*	−0.4	5.7*	6.0*
	nF → F	−6.5*	−7.0*	−2.4*	−0.7	3.9*	6.1*
Female I	F → nF	−4.1*	−4.6*	−4.2*	−1.2	0.5	1.2
	nF → F	−6.4*	−6.7*	−3.4*	−0.5	3.8	4.8
Female II	F → nF	−5.3*	−6.0*	3.4	−1.2*	8.3*	10.0*
	nF → F	−7.1*	−9.2*	3.5	−2.3*	11.0*	13.6*

A, no change in exposure conditions; *B*, change in exposure condition as indicated. NM, not measured. * $P < 0.05$.

TABLE 4. *Analysis of variance of parameters with time*

	F_{time}					
	RBC	Hct	Hb	MCV	MCH	MCHC
Male I	4.00*	3.39	NM	0.05	NM	NM
Male II	65.36*	74.96*	13.39*	2.91	13.01*	17.21*
Female I	6.39*	5.42*	8.38*	2.46	0.94	1.76
Female II	13.25*	19.45*	5.59*	16.60*	21.45*	25.35*

Analysis of variance with treatment yielded no significant differences. NM, not measured. * $P < 0.05$.

made (1:67,600) for red blood cell concentration (RBC) and hematocrit (Hct) measurements.

For Hb, hemoglobin iron was oxidized by ferricyanide to form methemoglobin that was reacted with potassium cyanide in Drabkin's reagent to form cyanomethemoglobin. This product was measured optically at 540 nm with a dual beam photometer, and the result was expressed as hemoglobin/100 ml blood. RBC and Hct were measured via the detection of conductivity changes caused by the nonconducting blood cells in the conducting medium. The changes were displayed as number of cells per cubic millimeter of blood for RBC and percentage of whole blood volume for Hct. Each parameter was measured three times and the mean value for each animal was used for all statistical analyses.

Mean corpuscular volume (MCV) (in μ^3), mean corpuscular hemoglobin (MCH) (in pg), and mean corpuscular hemoglobin concentration (MCHC) (in %) were computed. For statistical analysis these parameters were treated as independent variables.

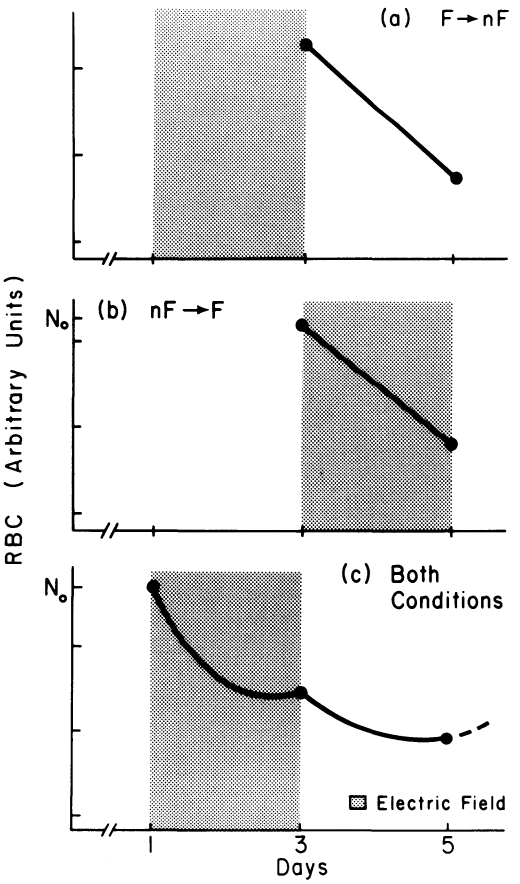

FIG. 1. Analysis of RBC results. *a* and *b*, Relationship between treatment and RBC in each experiment; *c*, interpretation of results. N_0, normal value. A similar analysis applies to HCT and computed indices.

RESULTS AND DISCUSSION

We chose to obtain blood via the opthalmic vessels because it was the least traumatic method by which each mouse could be sampled twice within a 2-day period. But it was still necessary, before applying the field, to determine the influence of the first blood collection procedure on the values measured after the second such procedure. We measured the blood parameters in two groups of mice, one male and one female, under conditions that were identical in all respects to those employed during the field-exposure portion of the study. The method of blood collection had a tendency to produce higher RBC, Hct, and MCV values and lower values of Hb, MCH, and MCHC (Tables 2*A* and 3*A*).

The results obtained in connection with the application of the electric field are shown in Tables 2*B* and 3*B*. In each experiment, RBC on *day 5* was significantly less than on *day 3* regardless of whether the interval between *days 3* and *5* was an exposure period or a nonexposure period. A decline in Hct paralleled the RBC changes, but Hb showed no consistent changes. MCV showed a tendency to decrease, but the other computed indices both

increased, since the cell loss overshadowed any decrease in hemoglobin concentration.

The trends in the computed indices, and especially the changes in RBC and Hct, were opposite to those induced by our method of blood collection alone. It follows, therefore, that the applied electric field had a physiological impact. The unique feature of the observed responses is that, for each parameter, a change in the same direction occurred with both the F → nF and nF → F groups. An analysis of variance confirmed that in all four experiments there was an effect associated with time but not with the order of field application (Table 4). This indicates that the animals responded to the change in their electrical environment, not to the electric field itself.

A dose-effect relation is sometimes incorporated into evaluations of specific ELF bioeffects (9). But such an assumption is inapplicable to our results because the observed effects were not due to the magnitude of the field. The stress hypothesis, on the other hand, is consistent with both our results and those of Phillips (10). Taken together, the studies seem to indicate that field-related changes in blood parameters are time dependent and, at least for fields of 5-100 kV/m, that they reach base line in 15-20 days—the expected result for stressed organisms that have passed beyond the acute response phase. This analysis is illustrated in Fig. 1, using RBC. When the field was present on *days 1-3* but absent on *days 3-5*, RBC on *day 5* was less than on *day 3* (Fig. 1*A*). We found the same results when the field was absent on *days 1-3* but present on *days 3-5* (Fig. 1*B*). Our interpretation is shown in Fig. 1*C*. Initially the mice were in equilibrium with the ambient environment and the RBC measured then was the normal value. When the electric field was applied it produced a transient decrease in RBC. This activated the body's homeostatic mechanism and RBC began returning to normal. But the field was switched off before the adaptive process had been completed, and this initiated a second, superimposed transient decrease in RBC.

In summary, our erythrocyte results show that exposure duration as well as the particular exposure history are factors that can influence observation of the existence and magnitude of ELF bioeffects.

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