Effect of Electromagnetic Fields on Experimental Developmental Abnormalities in Amphibians

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Electromagnetic fields are shown not to reduce the viability of amphibian embryos for either a short-term or a long-term exposure. The number of abnormal embryos drops under the influence of electromagnetic fields (a 3-h exposure being the most effective). An increased tempo of embryo development is observed for all times of exposure.

Key Words: *electromagnetic fields; developmental abnormalities; amphibian embryos*

Experimental findings on the biological effects of direct and alternating electromagnetic fields (EF) are contradictory. The viability of *Drosophila* eggs has been shown to be reduced for a 2-day exposure to alternating magnetic fields (0.5-msec rectangular pulses, frequency 100 Hz, total spike activity 1.76 mTl) [8]. This regularity is also true for vertebrates. The growth of chicks is slowed after exposure to an alternating magnetic field (45 Hz, 0.14 mTl or 60 Hz, 0.12 mTl) [6]; a spike activity of 0.4-104 μTl triggers teratogenic disturbances in the intestine, nervous system, and heart and alters the processes of segmentation of the axial structures and differentiation of the circulation system in chick embryos [4,9].

Studies carried out on amphibians are scant. Changes in the structure of chromatin of the cell nucleus have been shown to occur for a 12-24-h exposure of frog blood to single bilateral pulses (frequency 40-70 Hz, spike intensity 2 mTl), which is, in the opinion of Chiabrera *et al.*, indicative of cell dedifferentiation [3]. Diverse developmental abnormalities have been found to be characteristic of *Xenopus* embryos exposed to alternating magnetic fields (frequency 20 Hz and 2 and 20 kHz; 10-15 mTl; duration of exposure from 5 min to 8 h) [10]. These studies aimed at examining the effect of EF on the healthy animal organism or a culture of its cells. No special studies have analyzed the effect of EF on experimentally simulated developmental abnormalities of vertebrate embryos.

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MATERIALS AND METHODS

Fertilized ova of ribbed newts *Pleurodeles waltlii* (Amphibia Urodela), obtained under laboratory conditions in the absence of hormonal stimulation of the animals, were used in the study. The method of rotating the cleavage-stage eggs was used for experimental simulation of developmental abnormalities of the embryos. The coat was removed from the newt ova at the stage of formation of two blastomeres, and the ova were mounted between two glass plates. Deformation did not exceed 1/4 of the egg diameter. The eggs were then turned animal pole down and exposed in this position for 8-9 h. During this period cleavage was completed and the early gastrula stage was reached. During this stage, the embryos were removed from the

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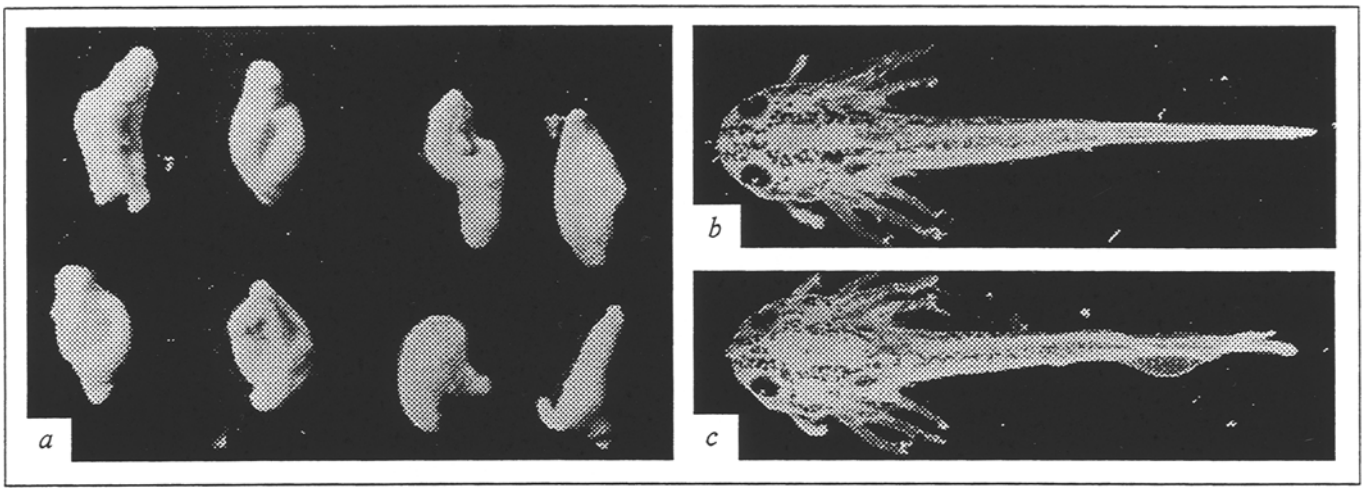

Fig. 1. Experimental developmental abnormalities in *Pleurodeles waltlii* and effects of EF. *a*) embryos of control group 5 days after simulation of abnormalities; *b*) larva of experimental group after hatching; *c*) larva of control group after hatching. Abnormal development of balancers and gills, changes of skin pigmentation, and caudal bifurcation noted in a control self-sustaining larva.

glass plates and divided into control and experimental groups. The experimental group was exposed to EF. For this purpose specially developed equipment (Volna Engineering Center, Moscow; Pat. № R-9201873, 04.06.92) was used, which created a modulated rotating EF with high-frequency signals in the range from 1 kHz to 1000 GHz and with low-frequency signals in the range from 0.001 to 100 Hz and made it possible to alter the angle between the electrical and magnetic components multivariately.

In all, we carried out three experimental series with different times of exposure of amphibian embryos to EF. The 1st experimental group was exposed to EF for 3 h and the 2nd for 18 h; the 3rd group was exposed three times to EF: for 3 h on the first day, for 18 h on the next day, and for 3 h on the third day. The development of the animals in the control and experimental groups lasted for 11-13 days, until the hatched larvae were self-sustaining. A total of 307 embryos were studied: 116 in the control group and 191 in the experimental. The animals were examined every 24 h in both the control and experimental groups.

In the course of the experiment and embryo development the embryos were photographed and fixed. Fixation was performed in a buffered formaldehyde (4%) after Lily. The embryos were dehydrated via dioxane and embedded in paraffin, and consecutive histological sections 10 μ thick were prepared. The sections were stained with hematoxylin and eosin after Mallory and embedded in balsam, and the state of the organs was morphologically investigated. An Ortholux 2Pol microscope (Leitz, Germany) was used for taking photos.

RESULTS

Our findings demonstrated that a 8-9-h exposure of the embryos in the upside-down position is sufficient to cause profound disturbances in their development. In the control groups the number of dead or inviable abnormal embryos constituted 96-97% (Fig. 1). The number of abnormal embryos in the control was approximately the same as the number of dead embryos. Alterations were most pronounced in the axial organs: in the notochord, in the spinal cord and brain, and in the branchial apparatus. Differentiation of the somites, heart, intestine, and protonephridium and migration of the neural crest cells were disturbed. Frequently the embryos were distorted and had neoplasms in the form of folds, outgrowths, or skin bubbles. Histological examination revealed various types of anencephaly, hypotelorism, brain hernia, disturbances of differentiation and migration of the embryonal cells in the nervous and excretory systems. In a number of cases cyclopy or the absence of the crystalline lens was observed. Approximately one half of the control embryos (50-60%) only attained the stage of the neural tube and stopped developing. Tissue disintegration then occurred and the embryo died. It should be mentioned that death resulted from exhaustion of the cell yolk deposits rather than from necrotization of the embryo. The development of surviving embryos was abnormal, with the above-mentioned defects. As a rule, such embryos were inviable forms which died at different times of development. From 7 to 8% of control embryos usually survived till hatching. However, these were incapable of moving around and eating properly. Only 3 to 4% of

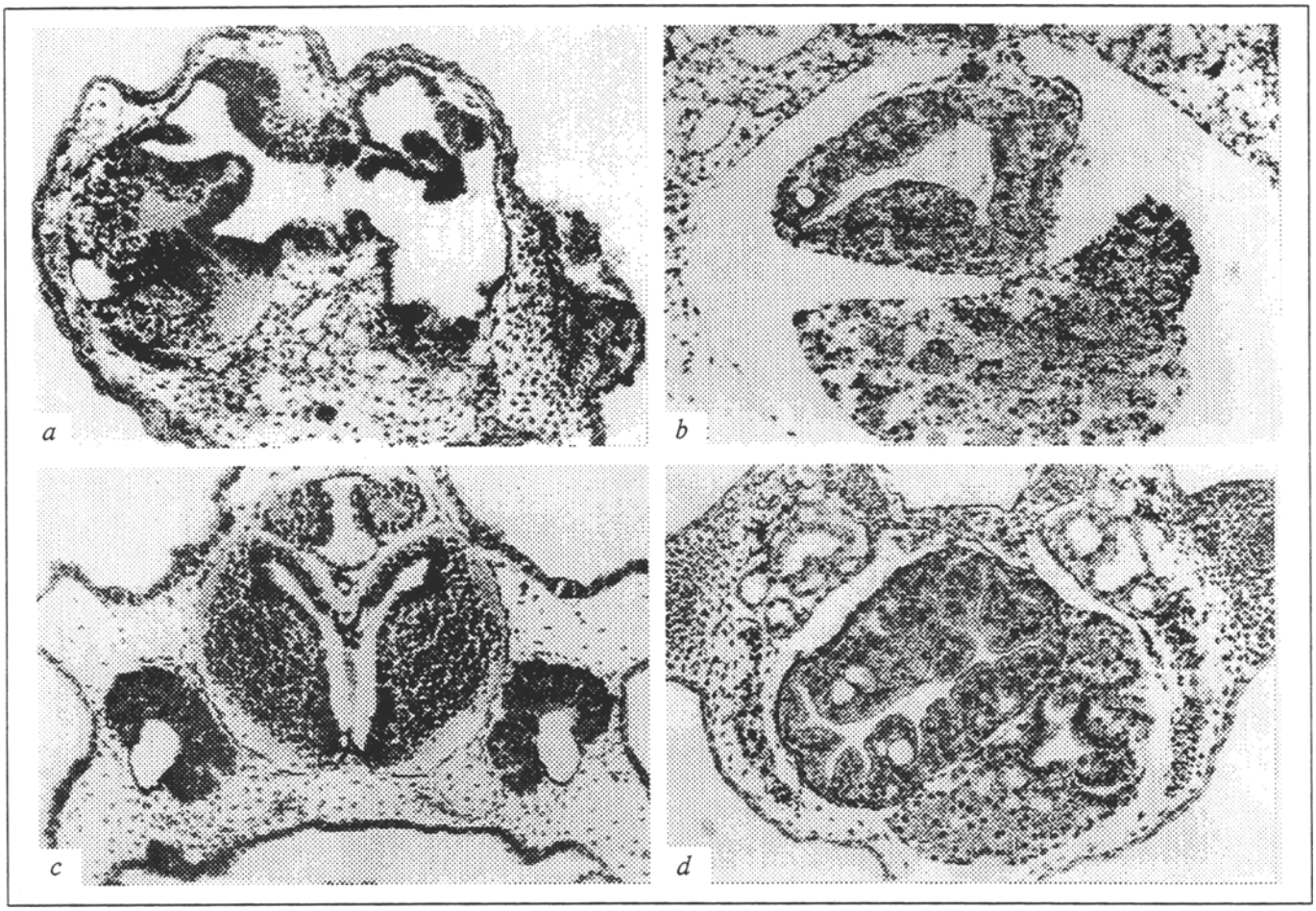

Fig. 1. Microphotographs of sections of larvae from control (*a* and *b*) and experimental (*c* and *d*) groups after hatching. *a* and *c*) sections through the forebrain and olfactory organ; staining after Mallory, ×240. *b* and *d*) sections through the intestine and excretory system. Enlargement of the internal cavity of the body and changes of the shape of the intestine and cell differentiation in the excretory system are noted; staining with hematoxylin–eosin, ×240.

animals preserved normal mobility and had a normal intestine. Despite their going over to active feeding, the majority of the larvae had minor skin defects or abnormal topology of the internal organs (Fig. 2). Histological examination of the animals in the control groups demonstrated that only 1 to 2% of them had no visual abnormalities in the structure of organs and cell differentiation.

The same abnormalities were characteristic of the experimental groups. Arrested development and mortality were uniformly typical of the embryos in both the control and experimental groups. This attests to the absence of teratogenic effects of the tested EF on the development of amphibian embryos. The experimental embryos died one day later than the controls, which indirectly corroborated the fact that pathological processes are delayed in the case of exposure to EF. It is to be noted that the effects of exposure to EF are diverse. Degeneration of abnormal embryos was retarded under the influence of EF, whereas the development of normal embryos was slightly accelerated in all three experimental series. Beginning from the 2nd day after exposure, the development of embryos in the experimental groups was 20-25 h ahead of that in the control groups. This difference slightly increased during the course of embryonic development and attained 30-34 h by the start of hatching. The effect may be due to nonspecific stimulation of the embryo metabolism under the influence of EF. Reportedly, an EF with an intensity of 0.8-6 Tl steps up the activity of enzymes (acetylcholine esterase, DNAase, cytochrome oxidase, catalase, carboxide dismutase, trypsin, and asparaginase) and RNA transcription [1,2,5,11].

A reduced number of abnormal embryos (40-45% lower than in the control group) was typical of all series of experiments. Meanwhile, the number of normal and viable individuals rose to 18-26%. Thus, the actual reduction of the fraction of abnormal embryos constituted 14-23%. However, marked differences resulting from different times of exposure to EF were discovered among the three experimental groups.

The number of dead embryos in the 1st experimental series was 2-3% higher than in the control. In the control the number of dead embryos peaked on day 3, whereas in the experiment this occurred on day 4. After a 3-h exposure to EF 26% of experimental animals (vs. just 3% in the control) had a normal morphological structure and were self-sustaining.

In the control the mortality of embryos was 2% higher than that in the 2nd series after a 18-h exposure. In the experimental group the mortality peaked on days 2 and 3 after exposure to EF. In the control the maximum mortality was observed on day 3 after exposure in the upside-down position, and 4% of larvae were able to be self-sustaining. In the 2nd experimental group 14% of the larvae went over to independent feeding, which was almost 50% lower than in the previous experimental series. In the 3rd series the embryos were exposed to EF many times. The mortality of embryos in the experimental group was 4% higher. We observed two peaks of mortality in this experimental group. The first maximum was between the 2nd and the 3rd exposure, and the second peak was detected after the 3rd exposure to EF. The maximum mortality in the control group was observed on day 3 after simulation of developmental abnormalities. The number of animals which were self-sustaining constituted 19% in the experimental group and 3% in the control, this being somewhat higher than in the case of a long-term single exposure to EF.

Thus, several specificities of the effect of EF on the development of amphibian embryos may be emphasized. First, the EF used by us do not reduce the viability of amphibian embryos for either a short-term or a long-term exposure.

Second, the number of abnormal embryos drops 14-23% under the influence of EF. A short-term exposure to EF (lasting 3 h) is most effective. The number of animals attaining the self-sustaining stage decreases by 5-9% for a long-term exposure to EF.

Third, an increased tempo of development (by 20-35 h as compared to the control) is observed for all times of exposure of the embryos to EF.

Studies of the effect of EF on the development of amphibian embryos with experimentally simulated developmental abnormalities suggest that EF of this type can be used for the prevention of early embryonal abnormalities.

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