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RADIOBIOLOGY AND RADIOECOLOGY

Autoimmune Process after Long-Term Low-Level Exposure to Electromagnetic Field (Experimental Results). Part I. Mobile Communications and Changes in Electromagnetic Conditions for the Population. Need for Additional Substantiation of Existing Hygienic Standards

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Abstract—Mobile communications provides a new source of electromagnetic exposure for almost the whole population of Russia. For the first time in the history of civilization, the brain of mobile phone users is exposed to localized radiofrequency (RF) electromagnetic fields (EMF). Base stations are a factor in the exposure of the population. Existing standards for limiting exposure do not account for the role of base stations as a source of EMF and cannot guarantee the absence of adverse health effects. It has become necessary to obtain reliable information to expand databases for the development of new standards. As recommended by the World Health Organization, an additional experiment is performed under the supervision of foreign experts, which shows changes in autoimmune status in rats after long-term low-level RF EMF exposure with an incident power of 500 μW/cm^2.

Keywords: radiofrequency electromagnetic fields, nonthermal intensities, autoimmunity, mobile communications, chronic electromagnetic exposure, hygienic standards

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AUTOIMMUNE PROCESSES AFTER LONG-TERM LOW-LEVEL EXPOSURE TO ELECTROMAGNETIC FIELDS (EXPERIMENTAL RESULTS)

of a study performed to confirm the results of Soviet studies conducted between 1974 and 1991 that showed the existence of immunological and reproductive effects from long-term low-level exposure of rats to radiofrequency (RF) electromagnetic fields (EMF). The previous studies were used, in part, for developing exposure standards for the USSR population. However, some members of the Advisory Committee of the World Health Organization (WHO) continue to ignore these results and do not use them in the development of international guidelines. Scientific debate on this matter continues to this day.

The WHO has decided to confirm the results of the USSR studies with the participation of independent scientific experts strictly within the framework of an international program.

In the current study, the conditions of RF exposure were made as similar as possible to those in the previous experiments: Wistar rats were exposed in the far field to 2450 MHz continuous RF wave with an incident power density at the cages of 500 μW/cm^2 for 7 h daily, 5 days a week over the course of 30 days.

Five of the papers in this journal present the results of the effects of the exposure on immunological parameters in the brain and liver of rats were evaluated using the Complement Fixation Test (CFT), as in the original studies, and an additional test, the more modern ELISA test. In addition, a pilot study was conducted to evaluate possible adverse effects caused by the injection of blood serum from RF-exposed rats on pregnancy and fetal and offspring development of rats using an animal model and protocol similar to that used in the previous Soviet study.

The results of the CFT and ELISA tests confirmed the findings of the previous studies and indicated possible effects on autoimmune processes caused by nonthermal RF exposure. The RF exposure resulted in increased formation of antibodies in the brain tissue extract but the exposure did not appear to be patholog-

Table 1. Total energy flux density (EFD) values of RF EMF of mobile communication base stations (220 stations)

Values	Energy flux density of equivalent flat wave, μW/cm^2
Average ($p < 0.05$)	+0.60 1.04 –0.38
Maximum	+74.59 128.60 –47.58

ical. Our results showed the same general trends as the previous studies suggesting possible adverse effects of the blood serum from exposed rats on pregnancy and fetal and offspring development in intact female rats.

The field of mobile communications is rapidly developing and mobile technology has a high rate of adoption in the daily life of the population. All layers of the population use mobile phones. Almost all people live in areas covered by wireless networks of different standards. Taking into account that the modern electromagnetic environment has been formed over the last 15 years and is still evolving (the appearance of third- and fourth-generation communication standards, Wi-Fi and TETRA systems, etc.), the evaluation of its possible health effects on the population is of great importance, as is the substantiation of policies aimed at preventing significant harm to the health of the population and adverse economic consequences.

MOBILE COMMUNICATIONS AS A SOURCE OF RADIOFREQUENCY ELECTROMAGNETIC FIELD (EFFECTS ON THE POPULATION)

Two elements of mobile communications systems represent hygienically significant sources of radiofrequency electromagnetic fields (RF EMF): mobile phones and base stations that receive and transmit signals.

Mobile phones emit RF EMF and impulse magnetic fields during calls. The many people who use mobile phones daily over long periods of time (years) expose their brains and the complex nerve structures in the inner ear, which ensure the normal activity of the auditory and vestibular analyzers, to electromagnetic radiation. The irradiation also affects the retina.

Base stations emit RF EMF 24 hours a day every day, and have over the past ten years or more subjected the entire population to constant exposure (including children, adolescents, adults, elderly people, pregnant women, developing fetuses, and ill and hypersensitive people). This irradiation has a dominant character, i.e., objective regularities and laws. RF EMC from base stations exposes the whole body to radiation.

Thus, the situation of global exposure of all groups of the population to RF EMF has arisen. Such exposure is predicted to be life-long for the current generations [1, 2].

Mobile communications creates a unique electromagnetic situation in the environment. The number of base stations increases each year as network operators strive for improved performance, thus increasing the overall EMF background in the environment. At the same time, the use of various technologies and standards of wireless communication has led to the appearance of numerous EMF sources, which operate in extremely close proximity to the population but emit EMF of significantly lower intensity compared to traditional sources of RF EMF, such as television and radio stations, for which the existing hygienic standards were developed. However, under modern conditions, the radiofrequency spectrum is used almost to its maximum extent, and every urban resident is exposed to RF EMF from multiple sources.

The existing standards do not take into account either this situation or the almost constant fractional effect of EMF on the brains of users, including children and adolescents. In our opinion, it is necessary to expand scientific knowledge by collecting data evaluating the possible influence of low-level RF EMF on the health of the population over the lifetimes of the current and future generations.

EVALUATION OF ELECTROMAGNETIC EXPOSURE FROM BASE STATIONS

The accumulating data on the electromagnetic situation in close proximity to mobile communication base stations make it possible to track its evolution through the development of wireless communication.

Before 2004, base stations were located at some distance from one another due to their relatively satisfactory throughput capacity. A sharp increase in the number of subscribers has led to a higher density of base stations together with a reduced capacity of individual units, which has altered the pattern of electromagnetic exposure of the population. The data of the testing laboratory of the Center for Electromagnetic Safety show these changes. Before 2004 the measurements were taken mostly at sites with a single base station. The main results of the measurements of RF EMF of 220 base stations in Moscow and Moscow region before 2004 are presented in Tables 1 and 2. The methods and measurement conditions are described in detail in [3, 4].

Over the last five years, measurements of EMF have mainly been conducted at sites with more than one basic station. This is a global trend that was outlined at the 14th annual meeting of the Scientific Advisory Committee of the World Health Organization “EMF and Health” in June, 2009. The previously developed instrumental measurement technique was adapted to these conditions and used in 2008 for mea-

Table 2. Total energy flux density (EFD) values of RF EMF of base stations in control zones (1345 points of measurements)

Values	Energy flux density of equivalent flat wave, μW/cm^2			
	building roofs where BS antennas are installed	building rooms where BS antennas are installed	buildings and structures located on the first and second lines of the built-up area relative to base station	adjoining residential area
Average ($p<0.05$)	+2.54 4.38 –1.62	+0.89 0.89 –0.45	+0.25 0.25 –0.13	+0.35 0.35 –0.18
Maximum	+74.59 128.60 –47.58	+3.02 5.20 –1.92	+1.86 3.21 –1.19	+1.11 1.92 –0.71

Table 3. Values of RF EMF created by mobile communication base stations of GSM standard (118 stations)

Radio transmitting facilities		Energy flux density of equivalent flat wave, μW/cm^2	
		maximum	average
Base station installed on the tower; measurements in residential areas	Object with single BS	0.58 +0.58 –	<0.17
	Object with two BS	0.60 +0.6 –	<0.17
	Object with three or more BS	0.90 +0.90 –	<0.17
Base station installed on the roof; measurements on the roof and in places of possible unlimited access	Object with single BS	63.19 +36.96 –	+1.8 73.19 –1.18
	Object with two BS	133.70 +78.20 –	+2.84 4.85 –1.79
	Object with three or more BS	244.10 +142.77	+3.57 6.10 –2.25

suring RF EMF of 118 base stations of the GSM standard in one of the regions of the Central Federal District of Russia. The results are presented in Table 3.

The highest values were recorded in areas where access is restricted, but where uncontrolled access by people is possible, and access by a variety of biological objects is also possible. Taking into account the measurement error, the highest measured value of energy flux density (EFD) of EMF was 386.87 μW/cm^2, which was formed by at least three base stations operating at different frequencies in the same range. The biological effects of this exposure regime are poorly studied for both humans and ecosystem elements in general.

Due to the relatively high altitude of their positioning and the characteristics of the radiation pattern of transmitting antennas, in most cases this type of source has no sanitary safety zone. The intensity of RF EMF created by base stations at the "earth level" in inhabited areas does not exceed the limit values.

The actual situation of exposure to modern sources of EMF in urban areas is represented by a nonstationary random process, which is characterized by a com-

plicated high-frequency regime combining continuous and intermittent modes of electromagnetic exposure. For this reason, to establish proper limit values, it is necessary to develop experimental techniques to account for the actual conditions of electromagnetic exposure of modern human beings.

ANALYSIS OF CHANGE IN ELECTROMAGNETIC BACKGROUND AND EXTENT OF POSSIBLE PUBLIC HEALTH RISKS

The RF EMF values in residential areas are relatively low and not dangerous to most groups of people according to the existing knowledge. We think, however, that it is necessary to demonstrate either the safety or the risk of long-term adverse effects on people under conditions of constant round-the-clock exposure to low-level RF EMF. It should be noted that certain important considerations make it difficult to evaluate this risk

First of all, the recommendations of the International Committee for Non-Ionized Radiation Protection (ICNIRP) were based on acute biological effects, rather than experiments on the effects of chronic exposure to RF EMF. Furthermore, the acute effects were identified on the basis of so-called thermal effects caused by high-level EMF exposure. The methodology used for the development of the Russian standards differs significantly: the biological effects of chronic exposure to low-level (nonthermal) EMF were studied. Thus, strictly speaking, the ICNIRP recommendations cannot be used for evaluation of the danger of long-term chronic exposure to RF EMF of nonthermal intensity because these recommendations were not based on criteria corresponding to the considered situation. Due to these substantial methodological differences, there is currently a significant gap between the Russian and international standards for the acceptable level of exposure of RF EMF in the range of mobile radio communications (10 $\mu W/cm^2$ vs. 1000 $\mu W/cm^2$, respectively).

The situation with Russian standards is equally complicated. The experiments with animals aimed at obtaining the basic data for substantiation of the limit values for RF EMF were carried out in 1970s. Animals were subjected to chronic RF EMF exposure for, as a rule, 1–4 months with EFD of 10–1000 $\mu W/cm^2$. The limit value of 10 $\mu W/cm^2$ for the population was initially set in 1984 and this value has not changed since. Since then, no further studies for establishing standard levels of RF EMF have been conducted in Russia. The given experiments did not consider a number of variables or factors: EFD values below 1 $\mu W/cm^2$ or complicated regimes of RF EMF exposure with simultaneous exposure to various frequencies; environmental factors that might alter the biological effects of EMF; the role of modulation; and the initial background of population health. In addition, base stations are also located in confined spaces with various configurations, e.g., in the metro, where it is difficult to evaluate the distribution of RF EMF due to the effect of reflection.

Thus, neither the existing Russian nor international standards for exposure to RF EMF from mobile communications base stations can guarantee that such exposure will have no adverse public health effects in the foreseeable future.

Taking into account the above considerations, the Scientific Advisory Committee of the international program “EMF and Health” of the WHO, at the suggestion of Russian scientists (Yu.G. Grigoriev), has decided to reproduce the data previously obtained in USSR on the biological effects of chronic exposure to low-level RF EMF. This problem is among the top-priorities of the WHO program. The WHO has endorsed the proposal to repeat the experiments on immunological effects previously obtained by the Kiev school (Shandala, Vinogradov, Rudnev, Dumanskyi, et al.) during the development of the currently existing limit values.

The authors of these publications [5–7] elaborated two models, which we used as the basis of our experiment.

Model 1:

– the experimental group of rats was exposed to EMF of 2450 MHz for a prolonged period of time (30 days) with EFD of 500 $\mu W/cm^2$;

– 7 and 14 days after completion of electromagnetic exposure, the animals were cut, serum was obtained from the blood, and antigens were prepared from the brain and liver;

– the Complement Fixation Test (CFT) was used to evaluate the extent of anti-tissue complement-fixing antibodies in blood serum to aqueous extracts of brain and liver tissues.

Model 2:

Blood serum of irradiated rats obtained 14 days after irradiation as described above was injected intraperitoneally into intact rats in the tenth day of pregnancy and then the rats were observed for pregnancy and fetal development and offspring fecundity.

The preparatory stage of the experiment started in 2005. The experimental program and protocol, with a detailed description of all stages of the study, were developed, and then confirmed with the WHO and an independent scientific advisory committee, including experts from the USA, Italy, and Germany. In agreement with the WHO, SSC Institute of Biophysics (now Burnasyan Federal Medical Biophysical Center, FMBA, Russia) was chosen as the base institution for the study. Irradiation of animals and dosimetry of RF EMF were conducted by the Center of Electromagnetic Safety.

The research group along with the advisory committee agreed on the conditions of RF EMF exposure, which were then adequately implemented. This was confirmed by dosimetric studies on the irradiation

stand performed together with specialists from France. The created conditions of electromagnetic exposure ensured a uniform radiation field for all groups of experimental animals in equal absorbed doses.

A group of specialists was chosen according to the profile of the planned study. The radiobiological laboratory of the Institute of Biophysics (headed by N. G. Darenskaya) was assigned to the work with animals during both the quarantine period (14 days) and the period of their exposure (30 days). This laboratory did not know the objectives of the experiment, thus providing double-blind conditions, as the subsequent work was performed by other researchers on encrypted experimental material.

The experiment started in September, 2006.

The whole cycle of the experiment including the processing of the obtained material, analysis of the results, and formulation of conclusions were performed with the active participation of the external experts from the advisory committee (J. Bushmann, Germany; C. Pioli, Italy; R. Sypnewski, USA) and with the active assistance of M. Repacholi, head of the WHO "EMF and Health" program.

The research team required three years (2005–2007) to complete the experiment according to the WHO protocol in the framework of the international program; the work was completed with much extensive effort and emotional strain.

The report on the experimental results and the general conclusions were endorsed by the WHO and the International Advisory Committee.

GENERAL CONCLUSIONS

(1) The study was conducted using the technique of the original experiments carried out in the USSR (G. I. Vinogradov and Yu. D. Dumanskyi, 1974, 1975). Both the original method based on the Com plement Fixation Test (CFT) and the modern immune enzymatic analysis (ELISA) were used to evaluate autoimmunity.

The biological investigations were carried out according to WHO recommendations.

(2) The results obtained by the CFT and ELISA methods confirm the data previously obtained in the USSR (G. I.Vinogradov and Yu. D. Dumanskyi, 1974, 1975) concerning the induction of the autoimmune response (the formation of antibodies to brain tissues) during chronic low-level RF EMF exposure (30 days, 7 h per day, EFD 500 $\mu W/cm^2$). Later, in 2004, analogous results were published by Ukrainian researchers [8] based on experiments under the following conditions of EMF exposure: 450 MHz, EFD 250, 500, and 1000 $\mu W/cm^2$, 2 h a day for 4 months.

(3) The experimental results confirm the data previously obtained in the USSR (M.G. Shandala, G.I. Vinogradov, 1982) showing the possible adverse effects of serum of rats irradiated with RF EMF (30 days, 7 h a day, EFD 500 $\mu W/cm^2$) on pregnancy and fetal and offspring development.

(4) The results of this work, along with those of other investigations concerning chronic low-level EMF exposure, can be used for the evaluation of the danger of RF EMF of base stations on public health.

More detailed data on the experiment, results, and conclusions are presented in the subsequent reports of (2, 3, 4, and 5) of this issue.

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RADIOBIOLOGY AND RADIOECOLOGY

Autoimmune Processes after Long-Term Low-Level Exposure to Electromagnetic Fields (Experimental Results) Part 2. General Scheme and Conditions of the Experiment. Development of the RF Exposure Conditions Complying with the Experimental Tasks. Status of Animals during Long-Term Exposure.

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Abstract—The paper describes the conditions for handling and exposure to radiation of the experimental animals (Wistar rats) and the methods of statistical processing of the obtained data, which were used to determine the effect of long-term exposure of the animals to radio-frequency electromagnetic fields (2450 MHz) of nonthermal intensity (500 μW/cm^2) on autoimmune processes; the study was performed under the auspices of the World Health Organization.

Keywords: radio-frequency electromagnetic fields, nonthermal intensity, autoimmunity, mobile communication, chronic exposure to electromagnetic fields, hygienic standardization

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INTRODUCTION

In the current work we describe the conditions for handling and exposure to radiation of the experimental animals; these conditions were used to study the effect of radio-frequency electromagnetic fields (2450 MHz) of nonthermal intensity (500 μW/cm^2) on the autoimmune status of the animals; the study was performed under the auspices of the World Health Organization.

EXPERIMENTAL

The long-term effects of radio-frequency electromagnetic fields (RF EMF) on the immunological status of animals was studied using 48 male Wistar rats from the nursery of the Russian Academy of Medical Sciences. The animals were in good health at the time of delivery (120–135 g). For a week, the animals were kept in quarantine and for another week, they were adapted to staying in anechoic boxes, where they were exposed to radiation. Teratology testing was performed on 120 rats (90 females and 30 males), which were kept in quarantine for two weeks.

The experiment started in September 2006. The animals were exposed to RF EMF with the energy-flux density (EFD) of 500 μW/cm^2 for 30 days (7 h per day). After each exposure, the rats were placed in a separate room of the vivarium at an air temperature of 21–23°C and relative humidity 40–60% under artificial light for 12 h per day, and at the air change rate of 100 m^3/h. Four rats were kept in each cage of 28 × 42 cm^2. The bottom of the cages was lined with a layer of sterilized sawdust of hardwood trees, which was specially designed for keeping small rodents. The cages were cleaned twice a week.

Feeding of the animals. The animals were given a standard dry food of the following composition: wheat; barley; maize; soybeans; wheat bran; soluble fish protein concentrate; milk powder; calcium carbonate; vitamins A, B_1, B_{2-6}, B_{12}, D_3, E, H, and K_3; and microelements Co, Cu, Fe, J, Mn, Se, and Zn. Food and water were not given during irradiation. The physiological control group, which was kept permanently in the vivarium, had free access to food and water around the clock.

The conditions of exposure to RF EMF of the experimental animals were similar to those used in several

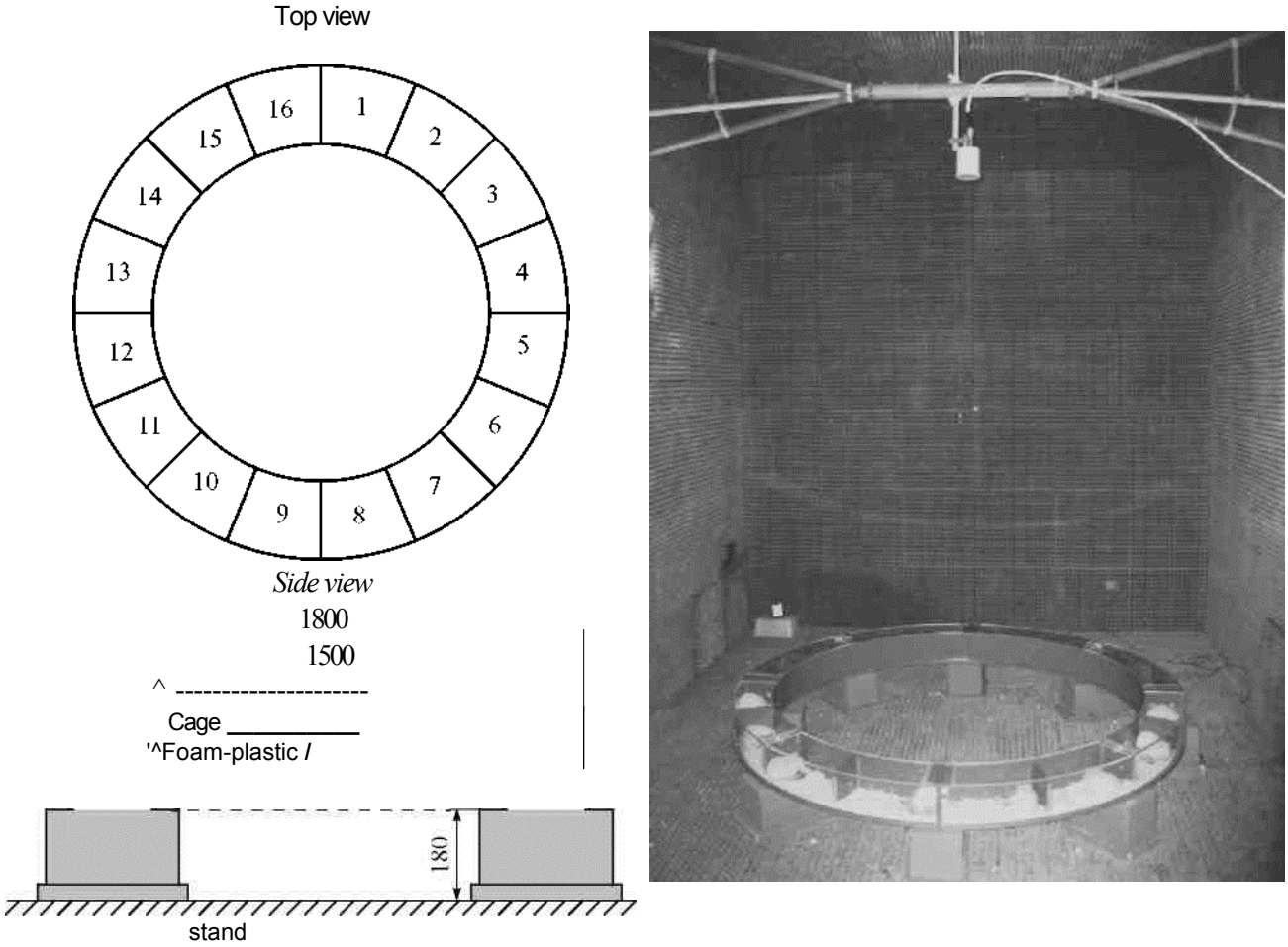

Fig. 1. The scheme of cage positioning and general view of the setup for rats exposed to irradiation in an anechoic chamber.

other studies [2–5]. The overall (total) impact of the elliptically polarized RF EMF of 2450 MHz (continuous generation) was effected in the far-zone field (plane electromagnetic field); the direction of influence was from the top. As mentioned above, the energy-flux density of the equivalent plane wave in the places where the animals were kept was 500 μW/cm^2; the mode of exposure was as follows: 7 h per day for 30 days, five days a week.

Both true and "sham" exposure to RF EMF were carried out in two shielded anechoic chambers. The walls, floor, and ceiling of the chambers were covered with a ferrite-based absorber with a pyramid-shaped working surface, 0.05 m high. The operating frequencies of the absorber ranged from 300 MHz to 15 GHz; the reflection coefficient was 15–20 dB for the entire range of frequencies. The outer surfaces of the chamber were made of welded steel sheets. The chambers had supply and exhaust ventilation. Chamber no. 1, where the animals were subjected to sham exposure, was about 10 m × 3 m × 3.5 m (L × B × H) in size; chamber no. 2, where the animals were subjected to true exposure, was about 6 m × 3 m × 3.6 m in size. To equalize the visual volumes of the chambers, a screen of black opaque material was installed in chamber no. 1. There was no natural light in the chambers. Artificial illumination was maintained in each chamber using six high-efficiency fluorescent lamps of 26 W power, with a color temperature of 4200 K (daylight). Prior to exposure, the following parameters were measured in both chambers: air temperature and relative humidity, rate of air change, illumination, electric field intensity, magnetic flux density within the frequency range of 5 Hz–30 kHz, and the power of the equivalent dose of γ radiation. The external conditions of the exposure were identical in both chambers. The EMF intensity within the frequency range of 5 Hz– 30 kHz and that of the γ radiation did not differ from the background values. The air temperature and relative humidity in the chambers were recorded immediately before and after animal exposure. In chamber no. 1, these parameters ranged from 20 to 21°C and 38 to 62%, respectively, whereas in chamber no. 2, from 20 to 22°C and 36 to 62%, respectively.

The experimental animals were kept in specially made radioparent ring-shaped (Fig. 1) cages from Atelier Deco Volume (Limoges, France). The cages were fabricated entirely of dielectric materials, organic

EFD of the equivalent plane wave at points corresponding to the geometric center of the cages at a height of 0.22 m above the floor level under conditions of "free space"

	Mean EFD values, μW/cm^2, including measurement error			
	measurement 1	measurement 2	measurement 3	the value averaged over three measurements
1	+287 494 −162	+285 491 −158	+287 494 −156	+286 493 −159
2	+292 503	+269 464	+275 474	+279 480
3	+295 509	+246 423	+270 465	−160 −172
4	+309 534	+275 474	+302 520	+295 509
5	+302 520	+274 472	+305 525	+293 506
6	+282 486	+297 513	+287 494	+289 497
7	+320 551	+296 510	+279 482	+298 514
8	+265 457	+283 488	+267 461	+272 468
9	+251 433	+265 458	+280 483	+265 458
10	+302 521	+261 449	+266 459	+276 476
11	+295 509	+267 461	+264 456	+275 475
12	+280 483	+288 496	+280 484	+283 487
13	+319 601	+326 562	+302 521	+326 561
14	+355 612	+341 589	+335 578	+344 593
15	+297 513	−198	+278 479	+295 509
16	+255 439	+248 427	+244 421	+249 429

glass, and PVC. The cage walls contained holes for ventilation. The "rings" contained 16 experimental animals which were not fixed, one rat per cage. The cages were covered with transparent lids. Each cage was set onto eight 0.18-m-high stands made of foam plastics. All 16 cages were similar in size, about 0.32 × 0.15 m. The bottom of the cages was lined with a layer (3–4 mm) of sterilized sawdust of hardwood trees, which was specially designed for keeping small rodents. The cages were cleaned daily after completion of exposure.

RF EMF was generated by a CMB-150-1 diather-mal-magnetronic apparatus Luch-11 (EMA factory; production of electronic medical equipment, Moscow, Russia) with a standard helical antenna 90 mm in diameter (tD5.861.003). The generator produced continuous electromagnetic waves at a frequency of 2450 ± 50 MHz; it was connected to an antenna feeder about 8.5 m long, which was made of a coaxial cable type RK50-11-21 with solid Teflon insulation. The antenna was mounted at a height of 2.35 m above the floor level in chamber no. 2 using a dielectric suspension made of plastics and wood only (Fig. 1). The output power regulator of the generator was adjusted to the position that provided an average power of 71.0 ± 7.3 W at the antenna input.

The mean EFD values of the equivalent plane wave were measured using a wideband Narda EMR-20 device (Pfullingen, Germany) connected by fiberoptic cable to a PC. The meter was equipped with an antenna converting the electrical field. The basic relative error of the measurements was EFD ±2 dB. In both chambers, the background integral mean EFD values did not exceed 0.17 μW/cm^2 within the frequency range of 0.1–3000 MHz at all points of measurement. The mean EFD of the equivalent plane wave were determined at points corresponding to the geometrical center of the chambers at a height of 0.22 m above the floor level under conditions of "free space," i.e., in the absence of rats and cages. The results of the measurements can be seen in the Table (the numbers of the cages are in accordance with Fig. 1). The EFD values ranged from 429 to 593 μW/cm^2 at an average value of 495 μW/cm^2.

RF EMF calculations performed by Leveque, an official expert on dosimetry (XLIM Laboratory, Limoges, France), demonstrated that the experimental setup provided EFD values of 500 μW/cm^2 within the cages, despite the fact that RF EMF was a little distorted by the "ring."

The specific absorption rate (SAR) characteristic of the given exposure conditions was evaluated using the method of finite-difference time-domain (FDTD). The digital rat model developed in the research laboratory of Brooks City Air Force Base (San Antonio, Texas, USA) consisted of 36 different types of biological tissues with resolution of 0.75 mm. The SAR value averaged over the whole body was 0.16 ± 0.04 W/kg at EFD of the incident field of 500 μW/cm^2. The SAR value averaged over the brain tissues was approximately 0.16 W/kg. The maximum SAR, 9.9 W/kg, was in the tail skin. The maximum SAR in the brain tissues was 1.0 W/kg. In addition, we studied the effect of changes in the relative positioning of the rats in the cages. There were small changes (about 15%) in the SAR values depending on the RF EMF polarization. The changes in the average SAR values at different rat positions in the cages did not exceed 5%.

The average EFD values of the equivalent plane wave were recorded in the reference point before switching on and after switching off the CMB-150-1 "Luch" generator. The stability of the generator's out-

put power was checked twice a week by measurements of the EFD values in the reference point for 7 h. The output power stability was satisfactory (within ±0.5 dB).

The behavior of the experimental animals during exposure to radiation was monitored using a web camera connected to a computer. The rats behaved calmly and were mostly sleeping. To ensure subsequent animal identification and studying within the "double blind" experimental framework, the rats subjected to true RF EMF exposure were marked with red using fuchsine, whereas the animals subjected to sham exposure were marked with blue using methylene blue (an area 10–15 mm in diameter on the rat's back was stained). The rats of the vivarium control group remained unmarked.

On the 7th and 14th days after completion of RF EMF exposure, the rat tissues were subjected to immunological and teratology tests. The results obtained are summarized in the following three reports.

Throughout the duration of exposure, the rat body weight was determined as an integral parameter of animal heath in all three experimental groups (the groups of true and sham exposure to RF EMF and the physiological control group). Figure 2 displays the results obtained.

Between the three groups, there were no statistically significant differences in the body-weight dynamics; however, the weight gain was naturally higher in the animals that had free access to food throughout the experiment (the physiological control group).

As judged from the dynamics of body weight and animal behavior in all three groups, the rats remained healthy over the three months of observation.

Statistical processing of the experimental data. The conventional methods of biostatistics were used in our study. In the immunological and teratology methods, the Student's *t* test, the non-parametric Wilcoxon signed rank test (*W*), and the non-parametric Mann– Whitney tests (*U* and *T*) were used to determine the significance of differences [6, 7].

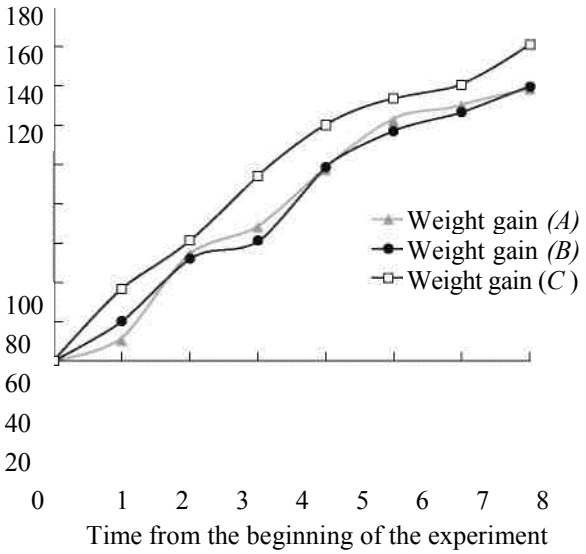

Fig. 2. Weight gain in rats of the experimental groups (*A*), (*B*), and (*C*) (exposure to RF EMF; sham exposure, and physiological control, respectively).

CONCLUSIONS

Thus, adequate conditions for exposure to RF EMF of the experimental animals, complying with the tasks of our study, have been developed. These conditions were appraised by foreign experts on dosimetry.

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RADIOBIOLOGY AND RADIOECOLOGY

Autoimmune Processes after Long-Term Low-Level Exposure to Electromagnetic Fields (Experimental Results) Part 3. The Effect of Long-Term Nonthermal RF EMF Exposure on Complement-Fixation Antibodies against Homologenous Tissue

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Abstract—The effect of long-term nonthermal EMF exposure of 2450 MHz (500 μW/cm^2) on the level of antibodies against brain and liver tissues is studied on rats by the complement-fixation test (CFT) in the cold. It is found that a statistically significant increase in the level of antibodies against aqueous extracts of brain and liver tissues in blood serum is detected only on the 14th day after exposure is ended.

Keywords: radio-frequency electromagnetic fields (RF EMF), brain and liver tissues, antibodies, complement-fixation test (CFT), polyclonal activation

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According to the data available, the effect of long-term nonthermal radio-frequency electromagnetic field (RF EMF) exposure results in the production of antibodies against brain tissue [1], changes in the antigenic structure of organs [2], autosensitivization [3–5], the formation of adaptive immunity [6], cellular degranu-lation of mast cells [5–7], and enhanced cytokine production [8]. However, most experiments performed were lacking a sham exposure group of animals, resulting in controversy in the interpretation of the results abroad and refusal to use the data obtained in the development of international regulatory documents for RF EMF.

It is necessary to specify the effect of nonthermal RF EMF on the level of antibodies against antigens of various organs (brain and liver) and reproduce the previously obtained results [1–5] using state-of-the-art methods, exposure conditions, and RF EMF dosime-try and with more precise experiment planning.

MATERIALS AND METHODS

The experiment was carried out on 48 male Wistar rats with initial masses of 200–220 g. The animals were kept under vivarium conditions. There were 3 groups of 16 rats each: the first group for cage-control, the second group for sham exposure, and the third group for nonthermal RF EMF exposure.

The rats were under total electromagnetic field exposure with frequency of 2450 MHz and with incident power density of 500 μW/cm^2 for seven hours daily over the course of 30 days.

The rats from all groups were injected intraperito-neally with 0.75 ml of 5% hexenal solution on the seventh and 14th days after the exposure ended. When the rat was anesthetized, within 1–2 min it was fixed on a special autopsy table with soft wires. The thoracic surface was painted with 5% alcoholic iodine solution, the thoracic cavity was quickly dissected with scissors, and the lungs and heart were removed. The blood which flowed into the thoracic cavity was collected using a sterile Pasteur pipette and put into a sterile test tube that was sealed with a stopper. In this way, 10– 15 ml of blood were obtained from each rat.

The obtained blood was put into a thermostat at 37°C for 30 min. Then the fibrin clot was separated from the tube's wall using a Pasteur pipette and put into a refrigerator for 30–60 min for better separation of the serum from the clot. After that, the clot was stirred with a glass stick and put into a refrigerator at +4°C for an hour.

The supernatant serum was collected using a sterile Pasteur pipette and put into a centrifuge bag, where it was centrifuged at 3000 rpm (2500 g) at 4°C for ten minutes in a K-24 D centrifuge with a fixed-angle rotor for complete purification from erythrocytes; it was then poured into Eppendorf tubes. After that, the

The level of antitissue antibodies against brain and liver tissues in various groups of rats

Antigen	Period after the exposure ended, days	Groups	Number of rats	Mean values	
				Me	log of the titer $M \pm m$
Brain	7	Cage-control Sham	5	<1 : 5	0.34 ± 0.21
		exposure RF EMF	5	1 : 5	0.68 ± 0.21
		exposure	5	1 : 5	0.68 ± 0.18
	14	Cage-control Sham	11	1 : 5	0.69 ± 0.08
		exposure RF EMF	11	1 : 10#	0.89 ± 0.05*
		exposure	11	1 : 20##	1.19 ± 0.07**
Liver	7	Cage-control Sham	5	<1 : 5	0.28 ± 0.17
		exposure RF EMF	5	1 : 10	0.66 ± 0.28
		exposure	5	1 : 5 <1 :	0.54 ± 0.23
	14	Cage-control Sham	11	5	0.06 ± 0.06
		exposure RF EMF	11	1 : 5	0.38 ± 0.11*
		exposure	11	1 : 5	0.44 ± 0.13*

Notes: * Statistically significant difference in comparison with the cage-control group, Student's criterion, $p < 0.05$. ** Statistically significant difference in comparison with the sham exposure group, Student's criterion, $p < 0.05$.
Statistically significant difference in comparison with the cage-control group, Mann–Whitney criterion, $p < 0.05$. ## Statistically significant difference in comparison with the sham exposure group, Mann–Whitney criterion, $p < 0.05$.

transparent, slightly opalescent serum was used in CFT according to the commonly accepted procedure [4]. It was kept in Eppendorf tubes at –18°C for three weeks.

To obtain antigens from brain tissue, the heads of intact narcotized rats were separated from the bodies along the occipital line. Two longitudinal sections were made from the occipital condyles to the eye sockets. The skull was dissected and the brain was removed from it. Then the abdominal cavities of the rats were dissected and half of the liver was removed, without the gallbladder.

The liver and brain tissues were shredded with scissors, purified from adipose and connective tissues, and purified from blood with physiological sodium chloride solution three times. Four parts by weight of physiological solution were added per one part of the organ. The obtained mixture was ground with a glass homogenizer. The homogenate was put into a tube and centrifuged at 2000 rpm (2000 g) for 20 min in an OPn-3UKhL 4.2 centrifuge. The supernatant liquid was poured into single-use plastic tubes for microsam-ples with a capacity of 1.5 cm^3. The tubes were sealed and kept in the frozen state at –18°C. The high-sensitivity CFT method in the cold was used [9–11]. The serums were defrosted and heated in a VEB MLW HRUFGF RATE-WERK ultrathermostat at 56°C for 30 min to destruct the proper complement. The heated serums were centrifuged at 3000 rpm (2500 g) for ten minutes in an OPn-3UKhL 4.2 centrifuge to remove the floc. Then the serums were used for CFT.

Before the CFT processing, the antigens were defrosted and centrifuged at 3000 rpm (2500 g) for ten minutes in an OPn-3UKhL 4.2 centrifuge to remove the precipitate floc. The supernatant liquid was poured into a tube and diluted with physiological solution in the proportion 1 : 10 to obtain the basic solution. It was titrated to determine the operating dilution of the antigen with no anticomplementary activity.

To carry out the CFT, the serum was diluted from 1 : 5 to 1 : 160. The operating dilution of the antigen (0.5 ml) was added to each tube. The tubes were put into the thermostat at 37°C for an hour and then placed in the refrigerator at 4°C for 18 h. After incubation in the refrigerator, 1 ml of hemolytic system, which was prepared by mixing equal volumes of hemolytic serum in 1/3 of the titer and a 3% suspension of sheep's erythrocytes, was added to each tube. The tubes were put into a thermostat at +37°C for 30 min. After that, the reaction was evaluated according to the following scheme:

++++ a sharply positive reaction and total hemol-ysis delay (the liquid is colorless with significant eryth-rocyte precipitation);

+++ a moderate positive reaction and a clear hemolysis delay (the liquid is pale pink with significant erythrocyte precipitation);

++ a positive reaction and a partial hemolysis delay (the liquid is hyperchromatic with erythrocyte precipitation);

+ a weakly positive reaction and insignificant hemolysis delay (the liquid is hyperchromatic with insignificant erythrocyte precipitation);

– a negative reaction and complete hemolysis (the liquid is hyperchromatic with no erythrocyte precipitation at the bottom of the tube).

The reaction with intensity ++ was taken as the CFT titer.

The anti-complementary properties of the serums and the anti-complementary activity of the antigen and its hemotoxicity were examined before the CFT. The serums and antigens taken in the used concentrations had no anti-complementarity or hemotoxicity. The following mean parameters were calculated when the obtained experimental data were processed: the median (Me) and the arithmetic mean of log of the antibody titer and its standard error ($\log M \pm m$). When the reaction was not detected in the initial dilution 1 : 5, the intensity was considered to be 0. The statistical significance of the discovered differences between the groups was evaluated by the Student's *t*-criterion for $M \pm m$ or the nonparametric Mann–Whitney criterion for the median [11, 12].

RESULTS AND DISCUSSION

The obtained data are generalized and presented in the Table. It follows from the Table that the level of antibodies against both used antigens in both test groups on the seventh day after the exposure ended was insignificantly different from the parameters of the cage-control group. The titer of antibodies against brain tissue in the cage-control group was Me < 1 : 5 ($M \pm m$ for log of the antibody titer was 0.34 ± 0.21), while the sham exposure group of rats and the one under the SHF EMF had Me of 1 : 5 ($M \pm m$ for log of the antibody titer was 0.68 ± 0.21 and 0.68 ± 0.18). The titer of antibodies against liver tissue in the cage-control group was Me < 1 : 5 ($M \pm m$ for log of the antibody titer was 0.28 ± 0.17), while the sham exposure group of rats and the one under the RF EMF had Me of 1 : 10 and 1 : 5 ($M \pm m$ for log of the antibody titer was 0.66 ± 0.28 and 0.54 ± 0.23).

The level of antibodies against brain tissue increased on the 14th day after exposure ended in comparison with the previous investigation period. The titer of antibodies in the cage-control group was Me = 1 : 5 ($M \pm m$ for log of the antibody titer was 0.69 ± 0.08), the sham exposure group of rats had Me = 1 : 10 ($M \pm m$ for log of the antibody titer was 0.89 ± 0.05), and the rats under the RF EMF had Me of 1 : 20 ($M \pm m$ for log of the antibody titer was 1.19 ± 0.07). The difference between the tested groups of animals was statistically significant.

The titer of antibodies against liver tissue in the cage-control group on the 14th day after the exposure ended was Me < 1 : 5 ($M \pm m$ for log of the titer was 0.06 ± 0.06). The sham exposure group of rats and those under the RF EMF exposure had higher antibody titers in comparison with the cage-control group, which were Me = 1 : 5 and 1 : 5 ($M \pm m$ for log of the titer were 0.38 ± 0.11 and 0.44 ± 0.13). The difference between the tested groups and the cage-control is statistically significant.

The antibody production after RF EMF exposure may result from polyclonal activation of normal anti-tissue antibody production or the organism's autoim-munization by tissue destruction products.

The polyclonal activation may result from stress or the activity of many bacterial products, polyelectro-lytes, and ionizing radiation [15]. According to our data, nonionizing RF EMF exposure may be considered a similar activity. The polyclonal activation is characterized by increased functional activity of lymphocytes of different clones and their involvement in immune response, which is necessary for antibody synthesis. This may be considered as a reflection of the stimulation of nonspecific resistance or normal antibody production, or as the first stage of the organism's autoimmunization in response to autoantigen formation [16].

The assumption made is confirmed by the aforementioned fact of increasing antibody level in the test groups (sham exposure and RF EMF exposure) in comparison with the cage-control group. Stress may be caused by the removal of the animals from the cage, their placement in the box for exposure, transportation to the irradiator and back, and hypokinesia during the process of seven-hour exposure, which is confirmed by data from the literature. Hypokinesia under space-flight conditions [13] or in laboratory experiments [14] leads to stress and increasing level of anti-tissue complement-fixation antibodies. The possibility of autoimmune reactions after RF EMF exposure is stated by data from sources by various authors. They showed changes in the antigenic structure of organs [2] and the development of autosensitization [3, 5] confirmed by increased levels of antibrain and antiliver antibodies in rat's serum after RF EMF exposure in comparison with the sham exposure group. The maximum possible antibody synthesis for brain tissue may be explained by its isolation in the process of embryo-genesis and its relative foreignness for the organism's immune system [16].

The obtained data agree with the results of previous investigations on the stimulating effect of RF EMF on antitissue antibody production [1–3]. However, this effect in our experiments resulted in a less intense increase in antibody titers as compared to the experiments of the authors cited above.

CONCLUSIONS

(1) The level of normal antitissue antibodies against brain tissue in the cage-control groups (log of the titer were 0.44 ± 0.13 and 0.69 ± 0.08) was higher than that

against liver tissue (log of the titer were 0.28 ± 0.17 and 0.06 ± 0.06) in both the first and second tests.

(2) Chronic nonthermal SHF EMF exposure brought the maximum increase in the level of antitissue antibodies against brain tissue on the 14th day after the procedure ended (log of the titer was 1.19 ± 0.07). The effect was statistically significant in comparison with the sham exposure group of animals (log of the titer was 0.89 ± 0.05) and the cage-control group (log of the titer was 0.69 ± 0.08). The level of antibodies against brain tissue was reliably higher in the sham exposure group than in the cage-control one.

(3) The level of antitissue antibodies against liver tissue increased insignificantly on the 14th day after nonthermal SHF EMF exposure ended; however, it was reliably higher in both test groups (log of the titer were 0.38 ± 0.11 and 0.44 ± 0.06) in comparison with the corresponding cage-control group (log of the titer was 0.06 ± 0.06).

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RADIOBIOLOGY AND RADIOECOLOGY

Autoimmune Processes after Long-Term Low-Level Exposure to Electromagnetic Fields
Part 4. Oxidative Intracellular Stress Response to the Long-Term Rat Exposure to Nonthermal RF EMF

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Abstract—Forty eight male Wistar rats with initial weight of 200–220 g were exposed to EMF of 2450 MHz frequency and 500 μW/cm^2 intensity for 7 h daily over a period of 30 days. On the seventh and 14th days after completion of exposure to the long-term EMF, enzyme immunoassay revealed a significant increase in the amount of specific antibodies of the IgM and IgG classes against the compounds yielded by interaction of amino acids with nitric oxide and its derivatives; tryptophan metabolites; azelaic acid; small-chain fatty acids; small-chain hydroxylated fatty acids; and palmitic, myristic, and oleic acids.

Keywords: electromagnetic fields of microwave frequency, immunoglobulins, antibodies, stress, reactive oxygen species, nitric oxide, amino acids, small-chain fatty acids, kynurenine pathway

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INTRODUCTION

In recent years, with the development of mobile communications, the study of radio-frequency electromagnetic fields (RF EMF) has become an urgent problem. Analysis of data on the impact of RF EMF on mammal cells, both in vivo and in vitro, provides no conclusive evidence of toxic, genotoxic, or carcinogenic effects [1]. The adverse effects of RF EMF are assumed to depend of the carrier frequency and signal type [2]. To resolve the issue, we need to know the response of mammal cells and the whole body to electromagnetic fields of microwave frequencies. Under extreme conditions, the cell stress response is manifested in the formation of signaling molecules such as reactive oxygen species (ROS) and nitric oxide ($NO^{\cdot}$). These compounds are necessary to support tissue and body activities because they are signaling molecules of the regulatory network of the cell which determine cell response to adverse impacts [3].

However, intense formation of reactive oxygen species and nitric oxide results in lipid peroxidation and accumulation of damage to DNA and protein molecules. High amounts of these compounds, as well as disrupted DNA and protein repair, lead to oxidative stress on cells. Long-term oxidative stress causes this effect by the presence of a thermal component during exposure to RF EMF [22]. Low-frequency magnetic fields may also cause oxidative stress [23].

Because of the aforementioned, it is important to know whether cells respond to the impact of microwave-frequency EMF by activation of stress reactions, in which ROS and $NO^{\cdot}$ modulate the regulatory proteins that trigger intracellular adaptive processes, i.e., whether EMF impact induces oxidative stress.

Some studies of molecular biochemical parameters after exposure to RF EMF have demonstrated changes in cell functioning, such as altered patterns of genome expression [10]; activation of intracellular nitric oxide production; and abnormal regulation of the $NO^{\cdot}$ substrate, arginine [11, 12].

Analysis of ROS and $NO^{\cdot}$, the products of lipid peroxidation, as well as heat-shock proteins, both in vivo and in vitro has provided no conclusive evidence of oxidative stress after exposure to RF EMF. Numerous studies have found no increase in the content of these biomarkers of oxidative stress [13–18]. Nevertheless, intense formation of some of the above compounds has been reported [19–21]. Some authors explain stress on cells. severe diseases, such as cardiovascular failure, malignant processes, diabetes, etc. [4–6]. Therefore, biomarkers, which enable the identification of oxidative stress in people, are now the objects of scientific research [7–9].

Table 1. Characteristics of the chemical compounds determined by enzyme immunoassay

Abbreviation	Chemical compound	Source of formation and pathological significance
C6-C8-C10-C12	Small-chain fatty acids	Products formed after membrane damage. Interact with other cell constituents
C6-C8-C10-C12 OH Pi	Hydroxylated fatty acids	Final products of lipid peroxidation. High-reactivity compounds that damage the surrounding molecules
PAL/MYR/OLE	Phosphatidylinositol Palmitic/Myristic fatty acids/Oleic unsaturated fatty acid	
MDA + 4HNE	Malondialdehyde + 4-hydroxynonenal	
NO_2tyr NOArg NOCys + NOBSA	NO_2-tyrosine NO-arginin NO-cysteine + NO-bovine serum albumin	Products yielded by interaction of nitric oxide and its derivatives with amino acids, both free and included in the make-up of proteins. Change the protein functions
NOMet + NOAsn + NOHis	NO-methionine + NO-asparagine + NO-histidine	
NOw + NOTyr AZE	NO-tryptophan + NO-tyrosine Azelaic acid {nonanedoic (azelaic) acid}	Products of oxidation and hydrolysis of unsatur-ated fatty acids. Change protein properties
3OH Kyn	3-Hydroxykynurenine	Tryptophane metabolites with neurotoxic properties
ANT/XANT/3OH ANT	Anthranilic and xanthurenic acids, 3-hydroxyanthranilic acid	
Kyn A QUINA	Ky nu re ni c a c i d Quinolinic acid	

In this study, we aimed at elucidating whether the signaling molecules ROS and NO˙ induce a stress response to long-term exposure to low-intensity microwaves in vivo.

EXPERIMENTAL

Forty eight male Wistar rats weighing 200–220 g were kept under vivarium conditions. The animals were divided onto three groups: (1) biocontrol; (2) sham exposure; (3) true exposure. The rats of group 3 were exposed to an electromagnetic field of 2450 MHz frequency and 500 μW/cm^2 energy-flux density for 7 h over the course of 30 days.

Blood serum withdrawn for the enzyme immu-noassay (ELISA test) was stored frozen at –70°C in 1-ml plastic tubes with lids. On the seventh day after exposure, 15 samples were used in ELISA (five sera from each group) and on the 14th day, 33 samples were used (11 sera from each group). Immunoenzyme analysis (ELISA test) was conducted using a conventional procedure [24] at the Institute of Immunology, FMBA Russian Federation. The IgA, IgM, and IgG antibodies against 15 antigens (Table 1) were determined. The double blind method was used. Coded sera were diluted 500 times in a buffer and poured into the plates for ELISA. The intensity of the antigen–antibody reaction was determined on a spectrophotometer from the optical density (OD) at 492 nm (the maximum of dye absorption) and at 620 nm (optical dispersion).

The results were estimated as follows:

$$OD_{samplre} = \frac{(OD_{492} - OD_{605})_{s1} + (OD_{492} - OD_{605})_{s}}{} - (OD \quad OD_{605})$$

$$_{492\ 605\ \text{blank}}$$

where OD is the optical density of the sample.

To confirm the specificity and sensitivity of the reaction, the plate quality was evaluated using known antibodies against known antigens. The experimental data were evaluated from the average parameters: median, arithmetic mean, and standard error ($M \pm m$) of the antibody titer. The statistical significance of the differences between the groups was evaluated using the Mann–Whitney test. Only the results obtained with samples having optical density (OD) higher than 0.1 units were used in the data processing.

RESULTS AND DISCUSSION

The antibodies (Ig A, Ig M, and IgG) against 15 chemical compounds were determined in our study (Table 1). Lipid peroxidation and disruption of their metabolism are known to occur during oxidative stress. Analysis of the total amount of IgM antibodies demonstrated that on the seventh day after exposure the amount of antibodies against small-chain fatty acids, hydroxylated fatty acids, and palmitic/myris-tic/oleic fatty acids (C6-C8-C10-C12; C6-C8-C10-C12OH; PAL/MYR/OLE) was significantly higher in the group exposed to microwave frequency EMF

Table 2. Total amount of antibodies of different Ig classes against chemical compounds in experimental rats exposed to microwave frequency EMF (medians, units of optical density)

Chemical compound	Groups	Number of observations	Time after irradiation	Ig class	
				G	M
C6-C8-C10-C12;	Microwave frequency EMF	15	7	0.254	362*
C6-C8-C10-C12 OH;	Sham irradiation	14		0.225	0.262
PAL/MYR/OLE;	Biocontrol	15		0.132	0.241
	Microwave frequency EMF	32	14	0.166	0.277
	Sham irradiation	33		0.151	0.276
	Biocontrol	31		0.141	0.249
NO_2tyr ; NOArg; NOCys + NOBSA; NOMet + NOAsn + NOHis; NOw + NOTyr	Microwave frequency EMF	30	7	0.179*	0.208*
	Sham irradiation	30		0.130	0.143
	Biocontrol	29		0.130	0.126
	Microwave frequency EMF	66		0.152	0.165*
	Sham irradiation	66		0.137	0.112
	Biocontrol	66		0.108	0.123
AZE	Microwave frequency EMF	5	7	0.142	0.309*
	Sham irradiation	5		0.128	0.200
	Biocontrol	5		0.145	0.212
	Microwave frequency EMF	11	14	0.188	0.311
	Sham irradiation	11		0.177	0.284
	Biocontrol	11		0.136	0.240
ANT/XANT/3OH ANT;	Microwave frequency EMF	5	7	<0.1	0.119*
Kyn A	Sham irradiation	5		<0.1	<0.1
	Biocontrol	5		<0.1	<0.1
	Microwave frequency EMF	11	14	<0.1	<0.1
	Sham irradiation	11		<0.1	<0.1
	Biocontrol	11		<0.1	<0.1

* Statistically significant difference as compared to the sham irradiation group, Mann–Whitney test, $p < 0.05$.

(0.362 units OD) than in the sham exposure group or in the biocontrol group (0.262 and 0.241 units OD, respectively). Later (on the 14th day), the difference between the groups disappeared. These data suggest that soon after exposure to microwave-frequency EMF, changes in cell lipids, which are caused by activation of oxidative reactions, appear in a proportion of animal cells of the appropriate group, in contrast to other groups. Among IgG immunoglobulins, antibodies against the aforementioned compounds were found neither on the seventh nor the 14th day after exposure.

Analysis for other products of lipid peroxidation (malondialdehyde and 4-hydroxynonenal), which are often used as markers of oxidative stress, was not informative because a low amount of antibodies against these compounds (less than 0.1 units OD) was determined in animals of all groups.

Numerous experimental and clinical observations testify to the fact that nitric oxide is a compound that strongly affects cell growth [25–27]. Interaction of $NO^{\cdot}$ with reactive oxygen species may result in the formation of compounds with extremely high activity, such as peroxynitrite, which cause damage to DNA and protein molecules [28]. In particular, $NO^{\cdot}$ interaction with tyrosine yields nitrotyrosine, which is a widely used biomarker of cell stress [6, 29]. Note that the formation of the nitrotyrosine amino acid residue may be important for transduction of the intracellular signal, which leads to expression of the transcription factor NF-κB [30]. Similarly, interaction of nitric oxide and its derivatives with other amino acids in protein molecules may result in the formation of nitrocysteine, for example. Many researchers assume that $NO^{\cdot}$ plays the main role in the induction of cell stress [31]. The ultimate effect of $NO^{\cdot}$ depends on the amount of this agent, the cell redox status, and the cell type. Because of the aforementioned, we tried to determine the content of antibodies against the products yielded by the interaction of amino acids with $NO^{\cdot}$ and its derivatives.

The data summarized in Table 2 demonstrate that in the group exposed to RF EMF, a more significant amount of proteins interacted with $NO^{\cdot}$ and its derivatives than in animals of the sham exposure group. The differences between the parameters of these two

groups were statistically significant. On the seventh day after exposure, the content of the IgG and IgM antibodies was 0.179 and 0.208 units OD in the group of animals exposed to RF EMF; 0.130 and 0.143, in the sham-exposure group; and 0.130 and 0.126 units OD in the biocontrol group.

On the 14th day after exposure, the content of IgM antibodies was 0.165, 0.112, and 0.123 units OD in the groups of animals exposed to EMF, the sham exposure group, and the biocontrol group, respectively. These three groups did not differ in the content of IgG antibodies on the 14th day.

Azelaic acid (Aze) is a product of oxidation and hydrolysis of unsaturated fatty acids. Aze is known to have antioxidant properties; an increase in the content of this compound may be caused by adaptive processes which protect the intracellular structures from the ROS effect [32, 33]. On the other hand, this compound may bind to lysine and thereby change the protein properties [34, 35].

On the seventh day after EMF impact, the content of IgM antibodies increased up to 0.309 units OD. In the sham exposure and biocontrol groups, the content of antibodies against Aze was 0.200 and 0.212 units OD, respectively. On the 14th day after exposure to EMF, the difference in the content of IgM antibodies against this compound was equal between the groups. Throughout the entire study, the groups did not differ in the content of IgG antibodies to Aze.

The kynurenine pathway is the main pathway of tryptophane degradation in mammalian cells [36, 37]. An increase in oxidative tryptophan degradation through this pathway leads to the development of neu-rodegenerative processes [38, 39]. A high level of sources of free radicals, such as quinolinic acid, 3-hydroxykynurenine, and 3-hydroxyanthranilic acid, results in a high level of oxidants in cells [40]. Activation of the kynurenine pathway of tryptophane metabolism is known to be often accompanied by oxidant stress. In patients with various chronic inflammatory processes, the amounts of both kynurenine and the products of lipid peroxidation were increased in the blood serum [41–43].

Because of this, it is important to study changes in tryptophane metabolism after exposure to the microwave-frequency RMF. It can be seen from Table 2 that on the seventh day after exposure to EMF, the amount of IgM antibodies against ANT/XANT/3OH ANT and KynA increased significantly up to 0.119 units OD. On the 14th day after the exposure, a negative reaction was recorded (<0.1 units OD). In the other two groups, the amount of these antibodies did not exceed the lower sensitivity threshold of the method either on the seventh or on the 14th days (<0.1 units OD).

Thus, our data suggest that long-term exposure to microwave frequency EMF (EFD 500 $\mu W/cm^2$, 7 h daily for 30 days) triggers the stress response of the organism. It may well be that irradiation causes the formation of antibodies against the compounds yielded by interactions of the reactive oxygen species and nitric oxide with intracellular molecules. The maximum effect was observed on the seventh day after exposure to microwave-frequency EMF. The intensity of the effect is reduced by the 14th day after irradiation. The antibodies against the compounds studied are mostly IgM immunoglobulins; less often they belong to the IgG class; IgA antibodies were not detected (in all cases, the sample optical density was lower than 0.1 units OD).

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RADIOBIOLOGY AND RADIOECOLOGY

Autoimmune Processes after Long-Term Low-Level Exposure to Electromagnetic Fields (Experimental Results) Part 5. Study of the Influence of Blood Serum from Rats Exposed to Low-Level Electromagnetic Fields on Pregnancy and Fetal and Offspring Development

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Abstract—The work evaluates the possible adverse effects on pregnancy and fetal and offspring development arising from the injection of blood serum from rats exposed to microwaves at a power density of 500 μW/cm^2 into intact female rats. The study is performed on 59 pregnant Wistar rats. Intrauterine mortality, embryo and fetal body weights, and placenta weight are used for the evaluation of embryo and fetal development. Generally accepted integral and specific parameters are used for the evaluation of the postnatal development of offspring during the first 30 days of life. It is shown that injection of blood serum from rats subjected to long-term RF exposure at 500 μW/cm^2 into intact rats on the tenth day of pregnancy results in adverse effects on fetal and offspring development. Higher total in utero and postnatal mortality, as well as delayed offspring development, are recorded.

Keywords: electromagnetic fields of low intensity, prolonged action, pregnancy, fetus, offspring
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In 1982, work [1] was published, giving an assessment of the autoallergic effects of the action of electromagnetic energy of the microwave range on the fetus and offspring; the work showed that the blood serum of MWF-irradiated rats (exposure to EMF 7 h/day for one month, PFD 500 μW/cm^2), injected into intact rats on the tenth day of pregnancy, negatively influences the course of pregnancy and fetal and offspring development.

The goal of the current work is to assess the possible damaging effect of the serum of rats subjected to prolonged RF EMF irradiation on the course of pregnancy and on fetal and offspring development.

MATERIALS AND METHODS

The study was conducted between October 20, 2006 and February 10, 2007 on 59 pregnant Wistar rats [2]. The serum-donor rats were subjected to RF EMF of PFD 500 μW/cm^2 for 7 h/day for a month. The pregnant rats were separated into three groups (Table 1). The first group of rats (21 females) was

Table 1. Distribution of rats by group and time of observation

Group	Characterization of group	Number of pregnant females	Time of serum injection	Distribution of rats by observation time		
				killing on 15th day of pregnancy	killing on 20th day of pregnancy	left to deliver offspring
1	Serum from EMF-irradiated rats injected	21	10	5	4	12
2		21	10	6	4	11
3	Serum from unirradiated rats injected (sham exposure)	17	– –	6	– 8	11
Total	Biological control	59		17		34

Table 2. Mass of embryos and placenta on 15th day of pregnancy with serum introduction on the tenth day of pregnancy

Group	Number of pregnant females	Number of live embryos per female $M \pm m$	Mass of one embryo $M \pm m$	Mass of one placenta $M \pm m$	Placentar coefficient $M \pm m$
Control	6	7.5 ± 0.9	151.1 ± 1.6	172.7 ± 2.2	1.14 ± 0.04
With sham exposure	6	8.3 ± 0.2	190.4 ± 5.4**	182.0 ± 5.9	0.96 ± 0.03*
With serum of irradiated animals	5	7.4 ± 0.4	185.4 ± 4.7**	175.9 ± 4.2	0.95 ± 0.04*

Notes: * Differences are statistically significant ($p \le 0.05$).
** Differences are statistically significant ($p \le 0.01$)

injected intraperitoneally with 1 ml of the blood serum of the EMF-irradiated rats on the tenth day of pregnancy. The second group (21 females) was injected on the tenth day of pregnancy with 1 ml of the serum from unirradiated animals—the group subjected to "sham" exposure. The biological control group consisted of the remaining 17 pregnant rats.

Five–six pregnant rats from each group were sacrificed on the 15th day of pregnancy (to assess embryo death and the characteristics of fetal development); four rats from each group were sacrificed on the 20th day of pregnancy to assess total intrauterine death; the remaining 11–12 pregnant rats per group were kept until they bore their offspring in order to assess the characteristics of offspring development and survival.

The characteristics of embryonic development were assessed by the indices of embryonic death and fetal and placental mass. Embryonic death was assessed by the formula

$$[C/(A + C)] \times 100\%, \quad (1)$$

where A is the number of live embryos and C is the number of resorbtions.

The condition of the offspring was studied during the first 30 days of life by a number of indices, including integral (fetal mass and death rate) and specific (appearance of fur cover, detachment of ear conch, eye opening, front tooth eruption, time of beginning of independent feeding) [3]. The indices were assessed according to their dynamic over the following periods: newborn, 7th, 14th, 21st, and 30th days. The symbol (+) was used to indicate the presence of the corresponding changes and (–) their absence. The number of offspring with positive reaction was compared to the total number of offspring. All of the indices were registered in an individual protocol of observations for every rat. In total, 133 newborn offspring were subjected to observation.

For statistical analysis, the confidence level of differences between groups of animals was determined using the Student's *t*-criterion. The error of the frequencies of events, which is connected to the probabilities (p) of embryo or offspring death expressed in percent, was calculated on the basis of formula

$$m = [p(100 - p)/n]^{0.5}, \quad (2)$$

where n is the number of animals in the group.

RESULTS AND DISCUSSIONS

Influence of Serum of EMF-irradiated Rats on Pregnancy and Fetal and Offspring Development in Intact Animals

Reaction upon serum introduction. A pronounced reaction 1 h after injection was observed in one rat from the sham exposure group (4.8%) and in three rats (14.3%) from the group injected with serum of irradiated rats. The character of the reaction in all animals was the same and was expressed in inertness; the animals lay curled up practically all the time and did not react to food and water. The reaction lasted for up to an hour. The reaction to serum introduction was observed in four animals out of the 45 experimental subjects (8.9%).

No deaths occurred during the time of the experiment among either the experimental or control groups of pregnant rats.

Period of embryonic development. The indices of embryonic development were assessed on the 15th day of pregnancy. The data presented in Table 2 indicate no differences between the groups in the number of live embryos per female (7.4 ± 0.4, 8.3 ± 0.2, and 7.5 ± 0.9 in the group injected with the serum of irradiated rats, the group injected with serum from the sham exposure group, and the control group, respectively). At the same time, the mass of the embryos in the experimental group and the sham exposure group significantly ($p < 0.01$) exceeded the mass of the embryos in the control group (185.4 ± 4.7, 190.4 ± 5.4, and 151.1 ± 1.6 mg respectively).

The mass of the placentas in the group of females injected with the serum of the irradiated rats did not differ from the mass of the placentas of the females injected with the serum of the sham exposure group or the mass of the placentas of the females from the control group (175.9 ± 4.2, 182 ± 5.9, and 172.7± 2.2 mg, respectively). The ratio of the placenta mass to the embryo mass (placentar coefficient) in the groups was 0.95 ± 0.04, 0.96 ± 0.03, and 1.14 ± 0.04, respectively. These data indicate some decrease in the placenta

Table 3. Mass of embryos and placentas in rats on 20th day of pregnancy and their viability

Group	Number of pregnant females		Number of fetuses		Average mass of fetus, g $M \pm m$	Average mass of placenta, mg $M \pm m$	Death of fetuses, % $M \pm m$
	total	delivered offspring	total	live off spring per female $M \pm m$			
Control	6	6	47	7.5 ± 0.8	–	637.5 ± 87.8	4.3 ± 2.9
With serum of irradiated animals	4	3	39	7.5 ± 0.8	3.7 ± 0.4	647.0 ± 83.2	23.1 ± 6.0**
With sham exposure	4	3	41	8.3 ± 0.7	3.8 ± 0.1		19.5 ± 6.0*

*Differences in relation to control are statistically significant ($p \le 0.05$).
**Differences in relation to control are statistically significant ($p \le 0.01$).

Table 4. Total intrauterine death of fetuses of rats in different observation groups

Observation time	Observation groups					
	control		with sham exposure		with irradiated serum	
	number of implantation places	number of resorptions	number of implantation places	number of resorptions	number of implantation places	number of resorptions
15th day of pregnancy	47		53		3	42 5
20th day of pregnancy	–	–	41		8	39 9
Females which did not give birth	–		–		–	70 70
Total number examined	47		94		11	151 84
Total death, % $M \pm m$	4.3 ± 2.9		11.7 ± 3.3		55.6 + 4.0***	

*** Differences in mortality indices of embryos in the group with introduction of serum of irradiated rats are statistically significant in relation to both the control and sham exposure groups ($p < 0.001$).

mass with respect to the embryo mass in the females of the experimental group and the sham exposure group.

In the group of females with introduction of serum of irradiated rats, embryo death on the 15th day of pregnancy somewhat exceeded the death in the sham exposure group and the control group (11.9 ± 4.6, 5.7 ± 3.2, and 4.3 ± 2.9 respectively). The confidence level for this difference between the groups was low.

Fetal period of pregnancy. Table 3 presents the data which characterize the indices of intrauterine development of the fetus on the 20th day of pregnancy in the groups of experimental animals. The materials of Table 3 show no statistically significant differences between the groups injected with the serum of irradiated animals and those injected with serum from the sham exposure group in the number of living fetuses per female (7.5 ± 0.8 and 8.3 ± 0.7 fetuses, respectively) or in the fetal mass (3.7 ± 0.4 g and 3.8 ± 0.1 g). At the same time, fetal death was higher in the first group than in the control ($p < 0.01$).

Assessment of total intrauterine fetal death on the 15th and 20th days of pregnancy in the tested females who did not give birth revealed a statistically significant (probability 99.9%, $p < 0.001$) higher level of fetus death in the group of females injected with the serum of irradiated rats (55.6 ± 4.0 %), in comparison with the sham exposure group and the control group: 11.7 ± 3.3% and 4.3 ± 2.0%, respectively (Table 4).

Rat fertility and newborn mass. The fertility of the rats, assessed from the number of live embryos on the 20th day of pregnancy and the number of live new-borns, did not differ between the sham exposure group and the control group (8.1 ± 0.7 and 8.2 ± 1.1, respectively). In the group of rats injected with the serum of irradiated rats, this index was 3.2 ± 1.1, a statistically significant difference at a 99% confidence level ($p < 0.01$) compared to the females of the control group and the sham exposure group (Table 5). There was no difference between the three groups in newborn body mass: in the group with serum from irradiated rats it was 5.5 ± 0.3 g; in the sham exposure group, 5.5 ± 0.2 g; and in the control group, 5.7 ± 0.3 g.

Offspring death up to the 30th day of life. Table 6 presents data on the death of rat offspring between birth and the 30th day of life. The materials from Table 6 indicate that in the group of females injected with serum from irradiated rats, the rate of offspring death as a percentage of the total number of offspring did not

Table 5. Fertility of rats from different observation groups

Group	Total number of pregnant	Killed on the 20th day of pregnancy			Delivery of young			Total number of newborns and live fetuses on the 20th day of pregnancy	Pregnancy	
		total	with live fetuses	total number of live fetuses	left to give birth	gave birth			number of fertile females	number of fetuses per female
						abs.	%			
With irradiated serum	16	4 4	3 3	30	12 11(1) 11	4(2) 9 11	33.3**	61	43.8	3.2 ± 1.1**
With sham exposure	15	–	–	33			90.0 100	122	80	8.1 ± 0.7
Control	11							90	100	8.2 ± 1.1 (1)

Note: (1) One female in group is not pregnant; (2) in eight pregnant females, fetuses were resorbed.

** Differences are significant in group with irradiated serum in relation to control and group of sham exposure ($p < 0.01$).

Table 6. Dynamics of death of rats from different group up to 30th day of life

Group	Number of born offspring	Death during observation process, days							Total death, %
		4	7	14	21	28	30	total	$M \pm m$
With irradiated serum	31	1	0	0	4	6	0	11	35.5 ± 8.6
With sham exposure	89	20	0	0	4	14	0	38	42.7 ± 5.2
Control	90	3	0	1	19	12	0	35	38.9 ± 5.1

Table 7. Total death of offspring in the period of intrauter-ine and postnatal life

Time of death	Observation groups		
	control $M \pm m$	with sham exposure $M \pm m$	with irradiated serum $M \pm m$
Total intrauterine death, %	4.3 ± 2.9	11.7 ± 3.3	55.6 ± 4.0*
Total postnatal death, %	38.9 ± 5.1	42.7 ± 5.2	35.5 ± 8.6
To ta l, %	43.2 ± 4.3	54.4 ± 4.1	91.1 ± 2.2*

* When comparing the death of embryos and total death of embryos and offspring, the differences are statistically significant in relation to both the control and sham exposure groups ($p \leq 0.001$).

differ significantly from the death rates in the sham exposure and control groups (35.5 ± 8.6, 42.7 ± 5.2, and 38.9 ± 5.1% respectively). In the control group, sham exposure group, and group with irradiated serum, offspring death mainly coincided (34, 20.4, and 32.3% respectively) with the end of the period of active milk feeding (after the 21st day of life) and the shift to independent feeding; these results correspond to population assessments of offspring death. It should be noted that in the group of females with serum of the sham exposure group, a high rate of early neonatal offspring death (before the fourth day of life—28.5%) was observed.

Comparison of the total death rate of offspring in the intrauterine and postnatal periods indicates a higher death rate of offspring in the experimental group at a very high confidence level of 99.9% ($p < 0.001$) in comparison with the sham exposure group and the control group (Table 7, Fig. 1).

It is also noteworthy that the share of intrauterine death of offspring differed between the groups. In the group of females injected with serum from irradiated rats, the share of intrauterine death with respect to total death was 61% (55.6 of 91.1%); in the sham exposure group, 21.5% (11.7 of 54.4%); and in the control group, 10.0% (4.3 of 43.2%). These data indicate a more pronounced embryotoxic influence of the blood serum of irradiated rats in comparison with the embryotoxic effect of the serum of unirradiated animals (in the sham exposure group).

Dynamics of offspring body mass. Offspring body mass in the experimental group was reliably lower than in the control and sham exposure groups. Delayed body mass growth in the offspring of rats injected with serum from irradiated rats in comparison with offspring of the control group and the sham exposure group is observed starting on the 14th day and increases with age (in the period from the 21st to the 30th day of life the confidence level of the difference rises to 99.9% ($p < 0.001$)). The greatest level of delayed growth is recorded in the period of the move to self feeding (Table 8, Fig. 2).

Some specific indices of offspring development. The data on the establishment of certain specific indices of

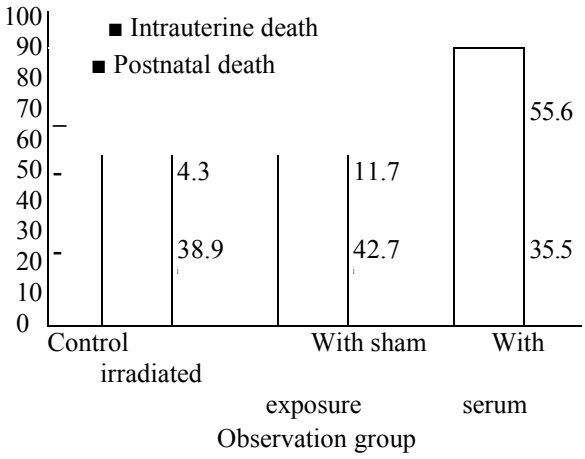

Fig. 1. Ratio of intrauterine and postnatal deaths of offspring in different observation groups.

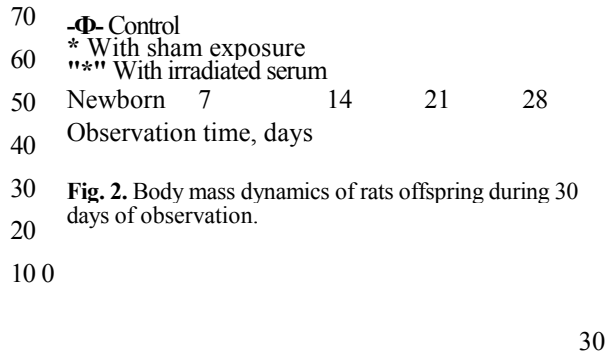

Fig. 2. Body mass dynamics of rats offspring during 30 days of observation.

offspring development, given in Table 9, indicate that in the control group in 96% of offspring the appearance of specific indices occurred at the time corresponding to the norm of physiological development. In the offspring of rats injected with the blood serum of rats irradiated with EMF and in the offspring of the sham exposure group, the appearance of the specific indices at the proper "physiological time" is registered in a significantly smaller number of animals than in the control group (75.2 ± 7.9 and 83.4 ± 4.5%, respectively). The number of offspring with normal indices in the group subjected to the serum of irradiated rats was significantly lower at a confidence level of 95% ($p < 0.05$).

The obtained data indicate that in the females subjected to the serum of irradiated rats, developmental delay of offspring is recorded more often than in the control group. The same is true of the sham exposure group, although to a lesser extent.

Study of the Mechanism of Influence of Serum from EMF-irradiated Animals on the Development of Offspring Using the Method of "Cross feeding"

The study was conducted on two groups of mature Wistar rats with "cross feeding" of offspring. The animals of the first (*I*) group were injected intraperito-neally on the tenth day of pregnancy with 1 ml of serum from EMF-irradiated rats. The rats of the second (*II*) group were not injected with serum—this was the control group. From each group, three pregnant rats at the same stage of pregnancy were selected.

After the rats gave birth, the offspring of the rats which had been injected with the serum from irradiated rats on the tenth day of pregnancy were moved for feeding to the control group, whereas the offspring of the control group were moved to the females which had obtained irradiated serum ("cross feeding"). Thus, the first group was not exposed to the direct influence of the EMF serum on intrauterine development, but during postnatal development was subjected to the immunological processes in the organism of the female (maternal milk factor) [4]. In the second group, the irradiated serum directly affected the fetus and the immunized organism of the female influenced intrauterine development, while this factor was absent in the postnatal period of life. To characterize the offspring development we used integral indices: the dynamics of body-weight growth and offspring death before the 30th day of life.

Offspring condition. In the control group of females, 23 live offspring were born in three litters with average body mass 5.6 ± 0.3 g. No stillbirths were registered in this group. In the group of females injected with the serum of irradiated rats on the tenth day of pregnancy, 26 live offspring with average body mass 5.1 ± 0.5 g were born. No stillbirths were registered. The infant rats were changed between the groups. Thus, the first group was composed of "irradiated" females and 23 control offspring and the second group included control females and 26 "irradiated" offspring (Table 10).

Offspring death in the groups differed both in the number of dead offspring and the dynamics of death before the 30th day of life. In group I of irradiated females feeding control offspring, a total of seven infant rats died (30.4%); in group II of control females feeding irradiated offspring, 16 infant rats died (61.5%) (Table 10). This difference is statistically significant ($p < 0.05$).

The dynamics of offspring death also differed between the groups. In the group of irradiated females feeding control offspring, death was observed only in the early period up to the fourth–seventh day of life. In the later period no deaths occurred. The death of offspring in the group of control females feeding irradiated offspring increased with the age of the infant rats. The majority of deaths were observed at the end of the observation period, from the 21st to the 30th day of life. All in all, 16 offspring died (61.5%) (Fig. 3).

Table 8. Dynamics of body mass of rat offspring up to 30th day of life

Observation time, days	Group					
	control		with sham exposure		with irradiated serum	
	number of offspring	body mass, g $M + m$	number of offspring	body mass, g $M + m$	number of offspring	body mass, g $M + m$
Newborn	90	5.7 ± 0.5	89	5.5 ± 0.5	31	5.5 ± 0.9
7	87	12.9 ± 0.6	69	13.5 ± 0.4	30	11.9 ± 0.5
14	86	21.3 ± 0.7	69	22.1 ± 0.9	30	19.0 ± 0.4*
21	68	33.6 ± 0.9	65	29.6 ± 0.7*	26	20.4 ± 1.2***
28	57	53.3 ± 1.2	51	58.6 ± 1.6*	20	36.7 ± 2.3***
30	55	63.1 ± 2.2	51	66.5 ± 1.8	20	47.5 ± 3.4***
Body mass growth coefficient to 30th day		11.1 ± 0.6		12.1 ± 0.7		8.6 ± 0.6*

* Differences are statistically significant ($p \le 0.05$); *** Differences are significant in relation to the control and sham exposure groups ($p \le 0.001$).

Table 9. Some specific indices of rats offspring development

Index	Group					
	control		with sham exposure		with irradiated serum	
	total	%	total	%	total	%
Appearance of fur cover on the fifth day	87	100	69	100	30	50
Detachment of ear conch	86	100	69	100	30	100
Opening of eyes on the 16th day	77	100	69	76.8	30	76.7
Escaping the nest on the 20th day	75	85.3	57	70.2	25	77.2
Beginning of self feeding	75	96.0	57	70.2	25	72
Number of offspring with normal characteristics of development, $M \pm m$	87	96.3 ± 2.2	69	83.4 ± 4.5*	30	75.2 ± 7.9*

* Differences are statistically significant ($p \le 0.05$).

Table 10. Dynamics of death of offspring from cross feeding

Group	Observation time, days							Total death, % $M \pm m$
	number of offspring	4	7	14	21	28	30	
I ("irradiated" females with control offspring)	23	5	2	0	0	0	0	30.4 ± 9.6
II (control females with "irradiated" offspring)	26	2	0	0	4	9	1	61.5 ± 9.6*

* The death of offspring in group II with irradiated offspring is statistically significantly higher than in group I ($p < 0.05$).

The results of cross feeding confirmed the possible negative influence of low-intensity RF EMF on fetal development and indicated the unfavorable influence of "toxic factors" of irradiated serum transferred by females to their offspring in their milk.

Body mass dynamics. The data on the body mass dynamics of the offspring up to the 30th day of life also indicate some differences in the coefficients of body mass growth with age in different age groups (Fig. 4). In the period from the first to the seventh day of life the coefficient of body mass growth of the control offspring fed by irradiated females (group I) was 2.2 (5.6– 12.3 g) and in the group of irradiated offspring fed by

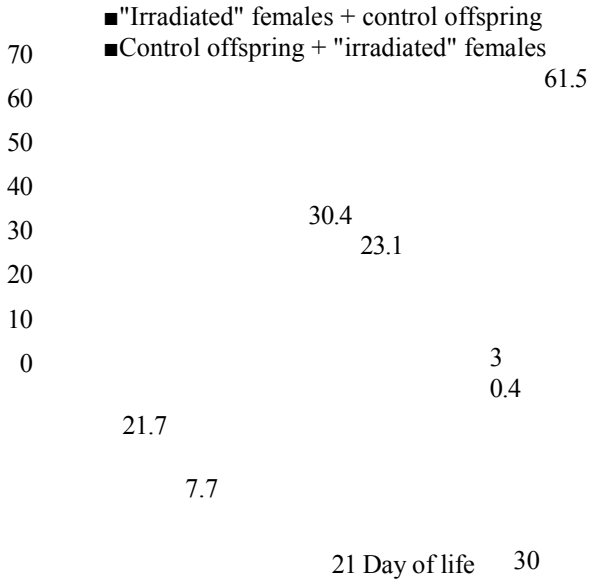

Fig. 3. Deaths of offspring in critical period of development in the groups of cross feeding.

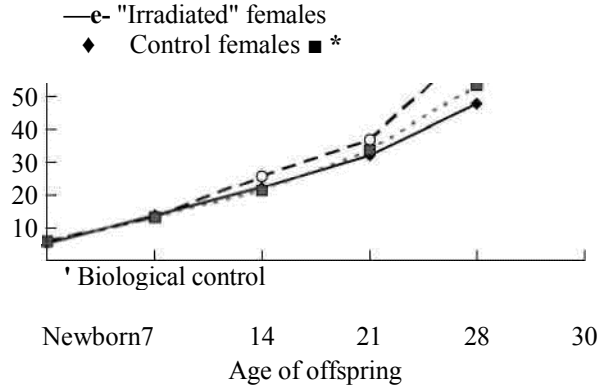

Fig. 4. Dynamics of average body mass of "irradiated" and control offspring.

control females it was 2.6 (5.1–13.2). It is noteworthy that the initial body mass of the offspring (newborns) was lower in the group of irradiated offspring moved to the control females. Beginning from the 14th day, differences in body mass growth were observed. In irradiated offspring fed by control females (group II), a deficiency in body mass growth in comparison with control offspring fed by irradiated females was observed.

Analysis of the results of cross feeding of irradiated and control offspring allow us to conclude that the processes (immune and other) which appear in the organism of females (mother) due to introduction of blood serum from irradiated EMP animals exert a pronounced embryotoxic effect on fetal development.

Thus, comparative analysis of the data indicate that the injection of 1 ml of blood serum of animals subjected to long-term exposure to RF EMF into rats on the tenth day of pregnancy negatively influences pregnancy and fetal development.

In the group of females which were subjected to the serum of irradiated rats on the tenth day of development, miscarriage is observed more frequently (66.4 ± 13.6%) at a confidence level of 99% ($p < 0.01$) compared with females injected with unirradiated serum (sham exposure—9.1 ± 8.6%) (Table 11). In the females injected with irradiated serum, there were cases of disturbances in the birthing process, which resulted in trauma to the newborns (11.1 ± 2.5%). Such disturbances were not observed in the females of the sham exposure group.

In the group of females injected with serum of irradiated donors, the total frequency of intrauterine death of offspring was higher (55.6 ± 4.0%) at a high confidence level ($p < 0.001$) compared with the sham exposure group (11.7 ± 3.3%) and the control group (4.3 ± 2.9%) (Table 12).

As we have already mentioned (see Table 7), in the group of females injected with serum from irradiated rats, the overall frequency of intrauterine and postnatal death of offspring is higher, at a high confidence level ($p < 0.001$), in comparison with the death of offspring of females from the sham exposure group and the control group: 91.1 ± 2.3, 54.4 ± 4.1, and 43.2 ± 1.4%, respectively; the coefficient of body mass growth is also lower: 8.6 ± 0.6, 12.1 ± 0.7, and 11.1 ± 0.6, respectively (Table 8).

In the offspring of females injected with irradiated serum, a higher frequency of delay in the establishment of physiological functions is observed in comparison with the offspring of females subjected to serum of sham exposure: 24.8 ± 5.5 and 16.6 ± 4.5% respectively, vs. 3.7 ± 1.9% in the control group (Table 9).

According to our data, the rate of fetal death in the intrauterine period was 55.6%, according to the previously published data this figure was 28% [1]; the indices of offspring mortality were 35.5 and 30.7%, respec-

Table 11. Summarized assessments of effects of influence of irradiated serum on pregnancy in rats

Index	Group	
	with sham exposure $M + m$	with irradiated serum $M + m$
Violation of time of giving birth, %	–	50.0 ± 11.2
Termination of pregnancy before birth, %	9.1 ± 8.6	66.4 ± 13.6**
Violation of lactation, %	30.0 ± 14.5	25.0 ± 21.7
Fertility, %	8.1 ± 0.7	3.2 ± 1.1**
Birth trauma (disturbances in birth), %	–	11.1 ± 2.5
Cannibalism, %	10.0	50.0
On average, %	17.0	33.3

Note: ** Differences are statistically significant ($p \leq 0.01$)

Table 12. Main indices of influence of irradiated serum on reproductive function of rats

Indices	Observation group		
	with irradiated serum $M + m$	with sham exposure $M + m$	control $M + m$
Fertility, %	43.8 ± 1.2***	80 ± 10	100
Embryonic death, %	55.6 ± 4.0***	11.7 ± 3.3	4.3 ± 2.0
	28 [1]	–	–
Number of fetuses per female	3.2 ± 1.1**	8.1 ± 0.7	8.2 ± 1.1
	2.6 ± 1.4** [1]	9.0 ± 0.8 [1]	7.9 ± 0.6 [1]
Body mass			
21 day	20.4 ± 1.2***	29.6 ± 0.7	33.6 ± 0.9
28 day	36.7 ± 2.3***	58.6 ± 1.6	53.3 ± 1.2
30 day	47.5 ± 3.4**	66.5 ± 1.8	63.1 ± 2.2

Notes: ** Differences are statistically significant ($p \leq 0.01$).

*** Differences are statistically significant both for control group and for group of sham exposure ($p \leq 0.001$).

tively, and female fertility was 3.2 ± 1.1 and 2.6 ± 1.04. Thus, the obtained results and previously published data on the influence of serum from EMF-irradiated rats on the development of offspring (Table 12) have revealed unidirectional changes in the indices of survival of offspring in the period of intrauterine and postnatal life.

CONCLUSIONS

(1) The injection of serum from animals subjected to long-term exposure to RF EMF (7 h/day for 30 days, PFD 500 μWt/cm^2) at a 1-ml dose intraperito-neally had a negative effect on embryonic development and fetal and offspring development.

(2) In the group of females with irradiated serum, in comparison to the sham exposure group and the control group, a higher level of embryonic death of offspring, lower fertility, and delayed physical development of offspring were observed.

(3) In the mechanism of the damaging action of EMF-irradiated serum on the development of offspring in intact animals, the most important role apparently belongs to the embryotoxic action of the irradiated serum.

(4) The results of the experiment in some respects confirm the previously obtained results (M.G. Shan-dala, G.I. Vinogradov, 1982) on the possible unfavorable influence of the serum of rats irradiated by EMF (exposure 7 h/day for 30 days to RF EMF of PFD 500 μW/cm^2) on the progress of pregnancy and development of the fetus and offspring.

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