REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From – To)*
11-08-2003	Final Report	01-Jul-02 - 11-Aug-03

4. TITLE AND SUBTITLE

~~Study of Repetitive HPM Effects on Individual Development of Drosophila~~

5a. CONTRACT NUMBER
ISTC Registration No: 2356

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5d. TASK NUMBER

5e. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

N/A

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

EOARD
PSC 802 BOX 14
FPO 09499-0014

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
ISTC 01-7006

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

20040625 086

14. ABSTRACT

This report results from a contract tasking Institute of High Current Electronics, RAS, Siberian Division as follows: While there have been many studies on the biologial effects of continuous-wave microwave energy, there is much less information on effects of pulsed energy, especially at high powers. Such information is needed, for example, to set appropriate safety standards for radar and communications systems. A key feature of pulses from such modern systems is their high electric-field strength, with the possibility of non-thermal mechanisms of biological interaction. Preliminary experiments in this lab with several simple organisms were suggestive of non-thermal mechanisms, but there were several methodologial issues that could give other explanations. Current research will follow-up on these issues and separate possible non-thermal versus conventional mechanisms. Also regarding safety, there have been almost no related studies on the development of individual organisms. This project will study of the biological effects of gigawatt, periodically-pulsed, nanosecond microwave radiation on individual development in the fruit fly, Drosophila melanogaster. Researchers will conduct dose-response, controlled studies at different stages of development and will determine the regularity and possible mechanism of any observed effects. The results of studies can be used as a basis for estimating hygienic and ecological dangers when operating such radiation sources.

15. SUBJECT TERMS

EOARD, Biological & Medical Sciences, Radiobiology

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18, NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Valerie E. Martindale
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On Sensitivity of Developing Organism of Drosophila to Repetitively Pulsed Microwave and X-ray Radiation

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The experiments with five-minute exposure by powerful microwave and (or) repetitively pulsed X-ray radiation (3 cm wavelength of microwaves with repetition frequencies 3-29 Hz, 2.4×10^8 W/kg peak SAR for nanosecond pulses, up to 50 W/kg average SAR; X-ray absorbed radiation doses up to 1.5 mRad/pulse) on Drosophila in embryo and post-embryo development stages were performed. As it was shown, such exposure leads to the interruption of development, the morphogenesis and the lifetime decrease. The percentage of interrupted development depends on the pulse repetition rate and the age of the exposed flies. The similarity of effects and the repetition rate dependence for the cases of high power microwaves and ionizing radiation is evidence of the analogous mechanism of action on the same cell targets, and among them bilayer lipid membranes, membrane-protein complexes of cells and, probably, nucleic acids are proposed. It is supposed that lifetime decrease may be due to the apoptosis triggering.

Key words: extremely high power microwaves, *Drosophila melanogaster*, interruption of development, apoptosis

INTRODUCTION

The pulse-repetitive microwave radiation of microsecond and sub-microsecond duration with pulsed power of up to megawatts is widely used in the communication systems, radiolocation and other areas of technique and as it's well known it can affect an operating staff at the violations of the safety standards. Usually, such type sources (magnetrons, for e.g.) have pulse repetition rate (p.r.r.) from several hundreds of Hz to kHz, and biological effects arisen are connected with thermal action (Schwan, 1957; Guy, 1984; Lin, 1978; Alekseev et al., 1997). Recent investigations (Pakhomov et al., 2000a, 2002) using powerful microwave pulses have shown that a number of physiologic effects to excited tissues are as well explained by the thermal action. It was O'Connor (1980) who paid serious attention to hazardous microwave radiation from the point of view of the teratogenesis of animals. Besides, it is known (Adey, 1980, 1981, 1993), that there exist effects of nonthermal nature dependent on the modulation frequencies. The actual concept does not except, that certain pulse repetition frequencies can represent the additional danger with respect to hygiene and ecology (Grigorjev, 1996). Our previous studies denote the large probability of anomalous development of Drosophila or the interruption (Bol'shakov et al., 2000, 2002a,b), when the exposure of developing organisms takes place in the range of pulse repetition rates from units of Hz to several tens of Hz.

To another class of pulse-repetitive microwave sources belong nanosecond pulse sources possessing extremely high power density in free space (Extremely High Power Microwaves – EHPM). For example, for physical researches or some applications there are produced the sufficiently compact nanosecond microwave generators based on high-current accelerators with e-beam pulsed power of 10^9-10^{10} W and gigawatt level of microwaves in the range of centimeter and decimeter wavelengths (Mesyats et al., 1990, Korovin et al., 1998). At that, electric field strength forward of the emitting antenna may be limited by effects of high-

frequency air breakdown or dielectric surfaces leveled to $E_b \approx 30$ kV/cm and above for nanosecond pulses, that corresponds to power level to 1 MW/cm^2 and some more. In spite of the high power density, in the nanosecond range of duration the energy flow density occurs to be relatively small in the view of the biological tissue heating. Just for typical fields penetration depth of the order of 2 mm (X-band) taking into account indispensable wave reflection (reflection factor from air-water boundary may comprise 50-70%) integral heating of tissues by one pulse turns out to be below 10^{-3} degree.

The use of single EHPM pulses (X-band, pulse duration of 10 ns, power flow of up to 0.3 MW/cm^2) and such objects as culture *E-coli*, mycelia fungus *Fusarium sp.* and *Drosophila* at different ages has not discovered deviations in the division rate or the interruption of development. The most noticeable was exposure of the pulse-repetitive radiation. Irradiation was carried out within 5 min, 15 min, and 1 hour (run of 5 min irradiation + 5 min pause) with fixed repetition rates of 6, 10, 16, 22, 50, and 100 Hz (Bol'shakov et al., 2000). At all repetition rates, there was no significant change in the cell culture division, whereas growth of *Fusarium sp.* colonies was slowing down during 1-hour cycle on average to 0.24 mm/hour (instead of 0.34 mm/hour) at all repetition rate values. Such a slowing down may be observed at the overheating by 20-25 degrees, while microwave heating of colonies on solid nutrient medium was essentially different in all range of frequencies, not exceeding 1 degree at the p.r.r. of 6 Hz. At certain p.r.r. the probability of *Drosophila* development interruption could make above 20%. Dependence on p.r.r. was non monotonic, for example, pupae (7 days aged) appeared to sensitive to p.r.r. of 22 Hz, and embryos of 1 hour aged were sensitive to p.r.r. of 6 Hz. In the last case, the filed out imagoes were increased death rate in the first days and sterility.

It was difficult to interpret results due to some reasons; one of which consists in that in these experiments along with microwave energy flow the objects were also affected by pulse-repetitive X-ray (roentgen) radiation. As is well known, at electron flow falling on a collector of the tube there appears a bremsstrahlung radiation with a wide energy spectrum of photons. This radiation filtering in the direction of vacuum antenna system is rather difficult. This circumstance is very important due to combined and simultaneous action of microwave and ionizing radiation (Grigorjev, 1987). That is why in the experiments mentioned above, all reasonable steps were taken in order to decrease X-ray doses using lead screens, labyrinths, and keeping objects distant from electron collector. The degree of such decrease was two-three orders due to distance kept and about two orders more due to lead screen near collector, so that the absorbed dose of ionizing radiation measured in the objects area leveled to 3×10^{-6} Rad/pulse.

According to data mentioned, the aim of this study was a detailed experiments and analysis of pulse-repetitive EHPM and X-ray effects on the developing organism of *Drosophila*, mainly in the post-embryo stages. In our plans there were measurements of the specific absorbed rate (SAR) in the immediate irradiation process and to obtain data for separate irradiation by microwaves using magnetron source and X-ray pulse-repetitive radiation with few doses.

This work was supported in part by the EOARD-ISTC grand.

MATERIALS AND METHODS

Subject of inquiry

The effect of influence on *Drosophila melanogaster* of *Canton S.* line was studied. The selection of samples was got by synchronized egg laying by flies on the nutrient media of standard composition: for 1 liter of medium 40 grams of manna-croup is boiled in water, and added are 40 grams of sugar, 40 grams of dry yeast, 20 grams of agar-agar, and 5 ml of propionic acid. The nutrient medium in volume as 4 ml was placed in glass vials each of 15 ml in volume. The number of eggs in each vial could vary from 10 to 40. Depending on exposure conditions (further (a)-(d)), the vials were placed into cassettes in groups of 14 – (a), (b), were situated in the waveguide one at a time – (c) or in the area of ionizing radiation by 2-4 pieces – (d).

Four ages of drosophila were used in experiments: larvae I, II, III (24, 48 and 72 hours

from the moment of egg clutching) and 7-day pupae. The objects were divided in three groups (amount of sampling at least 300 per group): exposure, sham exposure and control group. Alternatively, without irradiation, here were placed the vials with shame exposure objects. The control group was constantly kept at 24 ±1 °C.

(a) EHPM source: parameters and conditions of exposure

We used an X-band (central frequency 10 GHz) relativistic backward wave oscillator based on the pulse-repetitive electron accelerator SINUS-500, photo is shown in Fig. 1. The high-voltage unit of the source includes a pulse forming line with a built-in Tesla transformer, a spark gas gap switch, transmitting line, and a vacuum diode with magnetic insulation. The diode accelerating voltage in a pulse is 450 kV. An electron beam with 4.5 kA peak current and 20 ns duration (FWHM) is emitted from an edge-type tubular explosive-emission cathode and transported through electrodynamic system in external magnetic field (0.6 T induction). The magnetic field is produced by a DC solenoid with 16-kW power consumption. The microwave pulse power is no less than 200 MW, with ~7 ns width at FWHM (Fig. 2).

The microwave power is extracted in air via special electrodynamic system forming the basic (Gaussian) type wave beam in open space. The objects of irradiation were placed in ~1.5 m from the vacuum window of the emitting horn antenna. With that, the wave beam was turned for 90 degrees using a plane mirror. An element of microwave absorbing structure was used to support the vials with nutrient medium. The estimated strength of linear polarized electric field at the target was ~14 kV/cm and the corresponding power flow density was about 0.27 MW/cm^2. The estimation was made as follows. First, transverse distribution of microwave power density was measured in 1.5 m from the vacuum window of the antenna. The radial dependence obtained was interpolated by Gaussian curve. Then the caustic radius was found (where the electric field strength drops with factor of 2.718). According with the value obtained ($\rho \approx$ 21 cm) it was possible to calculate the spot diameter at a level of $0.7E_{max}$ (25 cm). The electric field strength in the beam axis was determined by the integral pulse power:

$$E_{max} = \frac{21.9}{\rho(cm)}\sqrt{P(MW)}\ ,\ kV/cm$$

The microwave pulse power was preliminary measured using both microwave detectors (calibrated by magnetron) and calorimeters with measured pulse energy 2 J. The electric field strength (14 kV/cm) measurement error was no worse than ±15%. This error is formed by the error of pulse power measurement (±20%) and the error of caustic radius measurement (±5%).

Note that the indicated electric field strength corresponds to the running wave and it's measured in the reflective object free plane where further the vials are placed. The walls of a vials and the nutrient medium itself create leakage fields, which are rather difficult to be calculated or measured.

(b) Conventional heating

In order to study effect of the high temperature, two water thermostats were used. In one of them the assigned temperature value could be maintained, and in the other one was the room temperature accurate within ±1 °C. The heating exposures were performed within 5 min by immersion of cassette with vials into heated water. At multiple heating exposures the interval was 10 min within which the cassette was immersed into the second thermostat. The temperature of nutrient medium in vials was measured by microthermistor MT-54 (Russia).

(c) Pulse-repetitive magnetron

The pulse-repetitive microwave irradiation of *Drosophila's* without X-ray component was performed by standard magnetron with frequency of 10 GHz and pulse duration of 330 ns at FWHM. The microwave pulse with a peak power of 40 kW was delivered to the target via standard waveguide of 23 mm×10 mm cross section. The microwave circuit (Fig. 3) included magnetron *1*, circulator *2*, attenuators *3*, and directed coupler *4*. The target chamber *5* consisted of adiabatic waveguide transit to the 72 mm ×34 mm waveguide and section with same cross size. In the end part of the chamber, heat-insulating support *8* and rubber microwave absorber *7*

were situated. The microwave power was monitored using directed coupler *4* and vacuum tube detector *11*. The detected pulse was recorded with oscilloscope *13* (TDS 220).

(d) X-ray sources

Two pulse-repetitive sources of the X-ray bremsstrahlung radiation were used. The first was an electron accelerator SINUS-500.

As for the case of X-ray radiation only, the irradiation process was performed for two groups of embryos simultaneously. The mentioned above irradiation scheme was implied for the first group (microwaves + X-rays), and the other group was placed into sealed metallic box which completely screened microwaves and was located nearby. The digital stand-alone dosimeter RAD-50 ("RADOS Technology Oy", Finland) was placed into the same box. The measurement of X-ray absorbed dose had shown 0.003 mRad/pulse (3×10^{-8} Gy). The waveform and energy spectrum of X-Ray pulses did not measured.

Of much interest was to perform pulse-repetitive irradiation by higher absorbed doses and to observe effect sensitivity to dose. With that end in view, the X-ray source on the basis of compact high-current electron accelerator SINUS-150 (peaking accelerating voltage is 270 kV, e-beam current is 4 kA at duration of 4 ns) has been prepared. The collector made of 1-mm thickness stainless steel and cooled by flowing water was used as X-ray converter in a planar diode with explosive emission cathode (2 cm diameter). The computer simulations by Monte-Carlo method and the program EPHCA2 were carried out. The conversion ratio of e-beam energy into bremsstrahlung with filtering taken into account was according to calculation of about 0.2%. The energy spectrum of photons is presented in Fig. 4. It is seen that in such spectrum the major part photons has energies of the range 80-160 keV. The calculated absorbed dose indirectly after collector did not exceed 10 mRad/pulse.

The absorbed dose could change by the distance from collector (L). The measurements were done using RAD-50 (at $L > 100$ cm), thermoluminescence dosimeter (Russia) and dosimeter VICTOREEN-541R ("Victoreen Quartz Dosimeters", USA). The two last devices provided dose measurements at $L = 5$ cm being the minimal length at which vials with *Drosophila*'s and nutrient medium were set. Here, the average absorbed dose was of 1.5 mRad/pulse.

For comparison with possible effects of continuous X-ray irradiation, the standard radiotherapeutic source of bremsstrahlung (RUM-17, accelerating voltage 210 kV, beam current 15 mA) was used. The standard testing dosimeters were placed in the irradiation zone.

Determination and evaluation of bio effects

The percentage of interrupted development (PID) was calculated by the number of larvae and pupae taken from each vial, as percent of not flying out imagoes with respect to the initial number of laying eggs in that vial. The average values of PID in all vials and the standard error mean were calculated. Besides, the observation of the flying out imagoes in point of view appearance of teratogenesis (body structure, sterility) was carried out. The intensity of death (Gavrilov and Gavrilova, 1991) in experiments with the pulsed X-radiation was registered.

Method of peak and average SAR estimation

The estimation of the peak SAR can be performed indirectly through heating of the upper layer of nutrient medium via the maximum temperature derivative (Pakhomov and Murphy, 2000): $\mathrm{SAR} = C\times(\mathrm{dT/dt})_{\max}$, where C is the specific thermal capacity of the medium assigned to 4.2 J/(g×K). Unfortunately, it is difficult to measure the temperature derivative during the nanosecond pulse. In fact, the approximation of adiabatic heating can be used when the value ΔT as the change of temperature per pulse of the width τ is defined. Then for the peak SAR we can write

$$\mathrm{SAR} \approx C\times\Delta T/\tau$$

Besides, if the value ΔT has been measured, in the case of the pulse repetitive exposure the average SAR can be determined:

$$\mathrm{SAR}|_{\mathrm{aver}} = C\times\Delta T\times f$$

Here f is a pulse repetition rate.

The earlier estimations of microwave heating of nutrient medium were based on the thermocouple method and the temperature measurements immediately after the irradiation (Bol'shakov et al, 1996, 2000). This method

gave the $SAR|_{aver} \approx 12$ W/kg at $f = 22$ Hz in experiment by using SINUS. The limitation of the method was that the temperature measurement could be started with certain delay (about 30 s) after irradiation and that is why it was a coarse estimate of the average SAR.

The correct estimate of an SAR in biological experiments with the electromagnetic exposure has a fundamental importance and one of the real variants of a good dosimetry connects with a real-time optical fiber thermometry (Pakhomov and Murphy, 2000a). We used this idea, purchasing the biomedical fiber-optic thermometer MT-04 (Russia). This device based on the temperature dependence of a luminescence decay time of the special glass material (the diameter of sensor envelope 1 mm) has the accuracy no worse than ±0.1 °C for absolute and ±0.01 °C for relative measurements. The temperature sampling period in these experiments is about 1 s (in the range of 0.6-1.7 s) and it is used for delivery of the light emitting diode pulses, also as to return a luminescence response into a photo detector. This time is quite enough to measure the temperature in a 1-mm water layer (estimated heat diffusion time about 8 s). The 12-m long fiber-optic cable allowed positioning of the thermo probe in the irradiation zone whole the device was situated in a screen-room connected with master computer. The sensor at the end of the fiber line was immersed in nutrient medium in 0.5 – 1 mm from the surface.

RESULTS

Temperature measurements

It was found by using SINUS-500 that a reserve of accuracy is achieved already for a 1-s batch of 100 pulses – Fig. 5. The arrows in the figure show the interval of the pulse batch. As can be seen, the increase in temperature after the batch (about 0.04 °C) exceeds the receptiveness. Higher deposition of microwave energy results in a more reliable monitoring of temperature rate (Fig. 6). Total results show that in a thin (~1 mm) near-surface layer of the nutrient medium is $\Delta T \approx 4\times10^{-4}$ °C per pulse. Hence, the peak SAR for $\tau = 7\times10^{-9}$ s can be estimated of 2.4×10^{8} W/kg and at $f = 22$ Hz the average specific absorbed rate is closed to 37 W/kg. It should be noted that the obtained values of an SAR may be low estimate (probably by 10-20%) due to the diffusion of heating in the intervals between temperature reporting. Another source of the error is a finite size of sensor. That is why we estimate the error of SAR absolute measurements as 50%.

It was of interest to compare measured value of ΔT with the estimation assuming that all the microwave energy flow (in open space) is converted to the heat. In this estimation, it is essential to know the effective depth for energy absorption. The skin depth was calculated for the water and estimated basing on the direct measurements of heating in various depth layers (by using the magnetron and an open waveguide end 23 mm×10 mm were used). The skin depth value made about 3 mm at 10 GHz. Thus, the effective absorption depth was about 1.5 mm and the energy flux from one pulse can be estimated as 1.9 mJ/cm^2. Then the maximum heating of 1.5-mm water layer should be 0.003°C per pulse. This value significantly (7 times) differs from the value measured. In our point of view, the cause of the difference is the large reflection of the electromagnetic wave from the glass vial and the boundary air-liquid. The special measurements of the reflection power from nutrient medium in a waveguide (23 mm×10 mm) show the value 0.65. It means that only one third of the power flux contributes to the heat.

The analogous temperature measurements for exposure by the magnetron were performed. It has been found that the maximal change of the temperature per pulse also reaches the value 4×10^{-4} °C. It follows that peak SAR on the magnetron reached of 5×10^{6} W/kg. The value of average SAR in pulse-repetitive mode of the magnetron is close to an $SAR|_{aver}$ value obtained on SINUS-500. It should be noted that the problem of non-uniformity of absorbed electromagnetic energy in the nutrient medium exists. This problem is typical for microwave irradiation of medium (and objects inside) with sizes exceeded the field penetration depth. Because of this an additional factor of statistically decreasing the resulting effect should be taken into account when larvae and pupae are situated over the total volume uniformly.

SINUS-500: bio effects

The exposures were made with a pulse repetition rate 22 Hz because this regime has been found earlier as most efficient on the embryo. The effect showed itself in the increased PID and appearance of the teratogenesis comparing with the sham exposure group (Table 1). In all cases the differences in data between EXP and SHAM groups are statistically significant with the probability not less than 95%. The disruptions of body segmentation were found for larvae I and II while there were no cases for larvae III, though larvae III was characterized by the highest PID. In fact, these results confirm the data of the first experiment (Bol'shakov et al., 2000).

To test the possibility of the effect accumulation, experiments with multiple (repeated) exposures were performed for larvae III and pupae that is the stages demonstrating highest sensitivity to single 5-min irradiation. It was found however that repeated exposures cause smaller effect than single exposure (Table 2).

Conventional heating: results

Drosophila larvae (3 stages) and pupae (7 days age) were put to the test at the increased temperature with three levels: 40, 50, and 60°C. Results were expected: the values of PID are very small and grow proportionally to the overheating in this range. The maximum PID was for 60°C and larvae III: PID = 2.8 ±0.8%. It is indicative that a thermal effect is much less than in case of the exposure to repetitive HPM pulses, despite the much higher overheating.

The special test on the multiple thermal exposures showed very effective accumulation of damages. In particular, eightfold 5-min heating of *Drosophila* embryo up to 55 °C practically in 100% cases the development was interrupted. In the case of EHPM the result was quite the contrary being related to the difference in character and targets damaged in cells.

Pulse-repetitive magnetron: results

The results of larvae and pupae irradiation at 22 Hz are presented in the Table 3.Maximum surface overheating per irradiation cycle was about 3 °C and less as 1°C in the depth of nutrient medium. The values of PID appeared to be reliably lower than in the case of using set-up SINUS-500. Nevertheless, as in embryo stages, it is necessary to establish a fact of the sensitivity of post-embryo stages to pulse-repetitive microwave radiation in absence of X-ray component. Noticeable is the fact that there were no cases of the disruptions of body segmentation fixed at using of magnetron (it is scheduled to perform experiments devoted to revelation of such anomalies as sterility and enhanced intensity of death of flying out imagoes).

X-ray sources: results

As it has been shown (Bol'shakov et al., 2000), the PID values for embryos 1-hour and 15-hours ages in case of combined exposure of microwaves + X Rays appeared in average comparable with corresponding values, microwaves excluding (in the case of X-ray only, the PID values were somewhat less at the same dependence on p.r.r. – 6, 10, 16, and 22 Hz). Of great interest occurred to be the obtained bio effect at the total absorbed dose within 5 min, (6-20) mRad, being proportional to p.r.r. of 6-22 Hz. The PID values at 6 and 22 Hz were reliably higher (5 % for 1-hour age and about 10% for 15-hour embryo, standard error was less than 2% at $p < 0.05$ in all cases), than for the group Sham and irradiated groups at p.r.r. of 10 and 16 Hz. Such bio effects have not yet been observed up to now, because of isotopic and continuous sources of X-ray are used as rule. In these cases, there is the low probability of bio effect at such dose (this dose is approximately 4 orders higher than the average ionizing radiation background for 5 min, and 3 orders less than the doses taken as small ones in the X-ray therapy).

Retesting embryos at p.r.r. of 3, 8, 13, 19, and 29 Hz has confirmed the effect. The minimum of PID values corresponded to p.r.r. of 13 and 19 Hz (below 2%).

Testing for other ages at the fixed p.r.r. of 22 Hz and absorbed dose 3×10^{-6} Rad/pulse was performed. The results are presented in the first column of the Table 3. In addition to the interruption of development, there were observed separate cases of disruptions of body segmentation (2.6% for larvae I) and the high mortality of flying out imagoes (for larvae II – 16% death

during the first day, for pupae – 60% death during three days).

Drosophila larvae I-III and pupae were irradiated at two values of absorbed dose 5×10^{-5} Rad/pulse and 1.5×10^{-3} Rad/pulse at p.r.r. of 10, 13, 16, and 22 Hz (results are in Table 4). More important results of this experiment in our point of view are following:

– PID value is increasing with a dose for the early post-embryo stages (see Table 4).

– More strong effect of the interruption in development corresponds to p.r.r. of 13 Hz when PID value can exceed 90% for the stage of larvae II and absorbed dose 1.5 mRad/pulse (Fig. 7). It is a maximum PID value either for PID dependence on age: PID(l-I) = 54.7 ±4.6%, PID(l-II) = 92.8 ±4.5%, PID(l-III) = 35.5 ±3.4%, and PID(p) = 24.3 ±4.7%.

– The pulse-repetitive X-ray exposure at p.r.r. of 13 Hz leads to essential shortage of the lifetime of flying out imagoes. Data for larvae I exposed are shown in Fig. 8 (the number of flying out larvae II, n=19, was not sufficient).

The experiment on the sterility testing of the flying out imago is being planned.

In a special experiment with the use of continuous X-ray source has shown that in the dose range of 3-25 mRad and 5-min exposure the PID value is practically constant being not dependent on embryos age and makes in average (4±1.8)%, ($p < 0.05$).

DISCUSSION

The enough strong bio effects on *Drosophila* clearly recognized by pulse-repetitive ionizing radiation with doses 0.05 and 1.5 mRad/pulse. At the dose level 0.003 mRad/pulse PID values are comparable with corresponding ones in the case of high-power microwaves at 5×10^{6} W/kg peak SAR, 330 ns pulse width. Regarding the bio effect of continuous ionizing radiation with the same total dose up to 25 mRad, it may be either intensive for all p.r.r. values and late post-embryo ages or weakened in p.r.r. range of 10-19 Hz and embryo stages. In both (HPM and X-Ray) cases PID values depend on pulse repetition rate and the age of group exposed. It seems, with the dose increase there appears some threshold effect resulting in effective growth of the number of unrepairable defects in cells and organism as a whole if *Drosophila* are irradiated at early stages of development and p.r.r. near 13 Hz. In addition to the interruption of development teratogenic events including high mortality are observed. The similar effect with much anomalies and mortality during the first days was earlier revealed for embryo stages at p.r.r. of 6 Hz combined irradiation HPM+X-rays (Bol'shakov et al., 2000). We can suppose that the active apoptosis (programmed cell death) is initiated similarly to the case of continuous irradiation of *Drosophila* by X-ray dose of 0.6 Gy (60 Rad) in the paper by Moskalyova and Zajnullina, 2001.

Presented in this paper the experimental results on *Drosophila's* may be systematized with respect to statements of non thermal effects of high-power pulse-repetitive microwaves.

- There is non monotone dependence of the PID for all development stages of *Drosophila's* on pulse repetition rate.
- The PID values at the microwave exposure with overheating of units of degrees can essentially exceed PID values at thermal heating as far as 60°C.
- The effect of injuries accumulation, as a characteristic for multiple thermal exposure, is absent at microwave exposure, moreover, reliable decrease of PID value is observed.
- There is similarity in irradiated embryo PID dependence on p.r.r. in cases of microwave and X-ray ionizing radiation.

The last statement is important because it should be taken into account that live system response to ionizing radiation is an example of the non thermal exposure.

Not having pretension of exhaustive explanation of the results obtained, several suppositions could be put forward. It is well known, the one of the basic aspects of ionizing radiation action, besides of the chromosome aberrations, is the accumulation effect of active radicals acting volatile both on genetic system (in our case it probably explains teratogenic consequences) and the number of other cellular systems. Among of systems opened to injury by excessive active radicals are lipid layers of mem-

branes and connected with them macromolecular complexes (enzymes). Just why cellular and mitochondrial membranes and related complexes are open to injury at shot pulses of RF fields and X-ray small doses? First, it may be initiating of some underground threat to cells, i.e. reactions of peroxide oxidation of lipids iteratively increasing the number of active radicals, and second, these organelles have rather large surfaces which actually increase the probability of reactions. According to the estimate, at X-ray dose of 1.5 mRad (that corresponds to the absorbed energy 0.1 eV/μm^3) in every cell about ten to hundreds of exited molecules, ions and free electrons can be formed. The fact of short pulse duration comparable with the typical lifetime of excited atoms (~10^{-8} s) should be important for the multiphoton processes, and, probably, it may play an important role in easing initiation of chain oxidative reactions (just due to the delay of response in the form of "defense" chemical reactions). Very like, though having its own peculiarities may be the microwave effect on lipid layers and membrane-active complexes. At first, a high strength of electric fields leads to the excitation of vibrational states of macromolecules by the dipole mechanism, so that the change of their potential energy may be considerable as compared to thermal energy. And second, the most of possible processes are specific, first of all, for the membrane-active complexes, and not only due to the high difference in complex permittivity of lipids and surrounding. It is necessary to take into account cooperative effects due to the dipole-dipole interaction and the intercoupling of the macromolecules through membranes, conformations and the change of lipid layers microviscosity. In addition to the processes mentioned, the existence of the high-excited states of atoms in the vicinity of membranes can provide the large tunnel ionization probability and the appearance of free electrons. Really, we must take into account the high strength of own fields of membrane lipid layers, and for such effect the applied RF field should not necessarily be comparable by amplitude. In essence, the appearance of charged particles and subsequent born of active radicals is, in our opinion, the most probable mechanism having common features in the cases of powerful microwaves and ionizing radiation.

About massaged damages of membranes also testify the results obtained in our cycle of experiments on inclusion of H^3 – thymidine into DNA of cancer cells mastocytoma P-815 and dependence of the DNA level on pulse repetition rate of microwaves by use the magnetron (Bol-'shakov et al., 2002b).

According to the results, the role of pulse repetition rate is the key one. And we could only suppose that damages are being increased or relaxed in the range of some frequencies due to native oscillation processes (mechanical or biochemical), or due to change in dynamics of the reparation mechanisms.

The effects differ on the age of *Drosophila's* irradiated, and it may be connected with the forming of reparation mechanisms on the some stages of development or with different cell growth rate when certain organs are formed. It is clear and corresponds to conclusions reported by Madhavan and Schneiderman, 1997. For example, for the ages of embryo and larvae I-II the intensive cell division of future lymphoid tissue, testicles and ovary takes place, and in the case of age of larvae II-III, brain.

In this paper, we have demonstrated the non thermal effects of high-power pulse-repetitive microwaves and X-rays on developing organism of *Drosophila*. The mentioned effects of powerful shot-pulse microwaves at repetition rates 6-22 Hz are not reproduced by conventional heating, but in many features are similar to effects of pulse-repetitive X-ray radiation.

ACKNOWLEDGMENT

The authors would like to thank academician G.A. Mesyats for the moral support of this work; Dr. M. R. Murphy and Dr. A.G. Pakhomov for the helpful discussions and comments.

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Table 1. Effects of SINUS-500 generator 5 min 22 Hz exposure in post-embryonic stages (EXP – exposed group, SHAM – sham exposure group). The magnitude of SAR is about 40 mW/g.

Stage	PID-EXP (PID-SHAM), %	Morphoses EXP (SHAM), %
Larvae I	13.7±1.3 (3.3±0.3)	3.4 (0)
Larvae II	14.8±1.7 (5.8±0.7)	1.5 (0)
Larvae III	24.4±1.8 (2.3±0.3)	0
Pupae, 7 days	23.9±3.5 (9.4±2.9)	0

Table 2. Effects of repeated (multiple) exposures in post-embryonic stages (EXP – exposed group, SHAM – sham exposure group).

Stage	Number of exposures	PID-EXP (PID-SHAM), %
Larvae III	5 min daily during 4 days	8,6±0,5 (2)
Pupae, 7 days	5 min daily during 4 days	7.0±0,3 (1.1)
Pupae, 7 days	6 times each 5 min during 1 hour	6.9±0,5 (1.2)

Table 3. Effects of magnetron generator 5 min, 22 Hz exposure in post-embryonic stages.

Stage	PID-EXP (PID-SHAM),%	Morphoses EXP (SHAM), %
Larvae I	10.5±1.2 (4.2)	0 (0)
Larvae II	10.6±2 (4.2)	0 (0)
Larvae III	18.1±3.3 (6.9)	0 (0)
Pupae, 7 days	15.5±3.1 (3)	0 (0)

Table 4. Effects of 5 min, 22 Hz X-ray exposure in post-embryonic stages.

Stage	$3*10^{-6}$ Rad/p EXP (SHAM),%	$5*10^{-5}$ Rad/p EXP (SHAM),%	$1.5*10^{-3}$ Rad/p EXP (SHAM),%
Larvae I	4.8 ±0.9 (3.3)	32.3 ±3.6 (10.8)	59.3 ±3.8 (10.8)
Larvae II	24.5±2.8 (5.8)	29.9 ±6.4 (10.8)	40.7 ±7.2 (10.8)
Larvae III	22.5±2.1 (2.3)	14.8 ±4.4 (10.8)	29.8 ±3.5 (10.8)
Pupae, 7 days	25.7±4.5 (9.4)	22.3 ±5.1 (10.8)	22.9 ±6.1 (10.8)

FIGURE LEGENDS

Fig.1. Photo of the relativistic backward wave oscillator based on SINUS-500 high-current electron accelerator. The peak microwave power is more than 200 MW at 10 GHz, pulse width 7 ns. The Gaussian beam power density at distance 1.5 m from vacuum window is of 0.27 MW/cm^2 in free space (without reflections). The peak SAR is estimated of 2.4×10^8 W/kg. Special microwave absorbers were used. Maximal pulse repetition rate of the source is of 150 Hz.

Fig. 2. Waveforms of vacuum diode voltage (1), electron beam current (2), and microwave detector signal (3). Time scale: 1 division – 10 ns; maximum diode voltage 450 kV; peak e-beam current 4.5 kA.

Fig. 3. Scheme of experiment on magnetron:
1 – magnetron generator, 2 – circulator, 3 – attenuators, 4 – directed coupler, 5 – waveguide chamber 72 mm×34 mm, 6 – vial with nutrient medium, 7 – rubber microwave absorber, 8 – heat insulator, 9 – light guide, 10 – fiber-optic thermometer, 11 – microwave detector, 12 – power source of the detector, 13 – TDS220 oscilloscope.

Fig. 4. Spectrum of bremsstrahlung in experiment on base SINUS-150 accelerator calculated by using Monte Carlo method in the program EPHCA2.

Fig. 5. Indications of fiber-optic thermometer MT-04 in batch microwave regime (100 pulses during 1 s). It is a fragment of data file which was obtained during temperature measurements of nutrient medium in the vial heated by EHPM on SINUS-500. The arrows indicate the beginning and cessation of the batch with the repetition rate 100 Hz. Such p.r.r. did not used for exposures of *Drosophila's*.

Fig. 6. Indications of fiber-optic thermometer MT-04 in batch microwave regime (6600 pulses during 300 s). The pulse repetition rate of EHPM is of 22 Hz.

Fig. 7. Dependences of the percentage of interrupted development for the stage of larvae II on p.r.r. for two absorbed doses of X-ray radiation. All exposures were performed by accelerator SINUS-150 at peak electron energy 270 kV, maximal e-beam current 4 kA with pulse width 4 ns. The data are presented as mean SEM ($p<0.05$). The number of larvae II in each group (EXP for dose 0.05 mRad/pulse, EXP for dose 1.5 mRad/pulse and SHAM group) was exceeded 300.

Fig. 8. Number of survival *Drosophila's* $\mu(x)$ to the age x, normalized to flying out imago at exposure on the stage of larvae I; p.r.r. is 13 Hz, X-Ray dose is 1.5 mRad/pulse. The number of flying out imagoes in EXP group was 142, in SHAM group 281. For the extrapolation of experimental data the Gompertz-Meikam law describing the death rate was used (Gavrilov and Gavrilova, 1997):

$I(x) = -\frac{d\ln\mu(x)}{dx} = A + R*\exp(\alpha x)$, where

parameters A, R, α were found by the least-squares method.

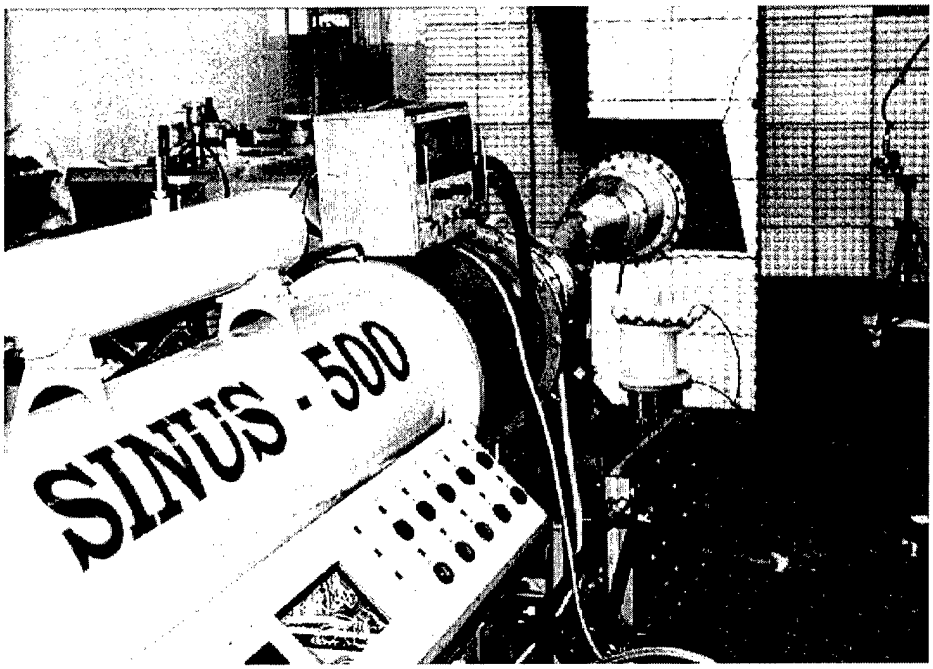
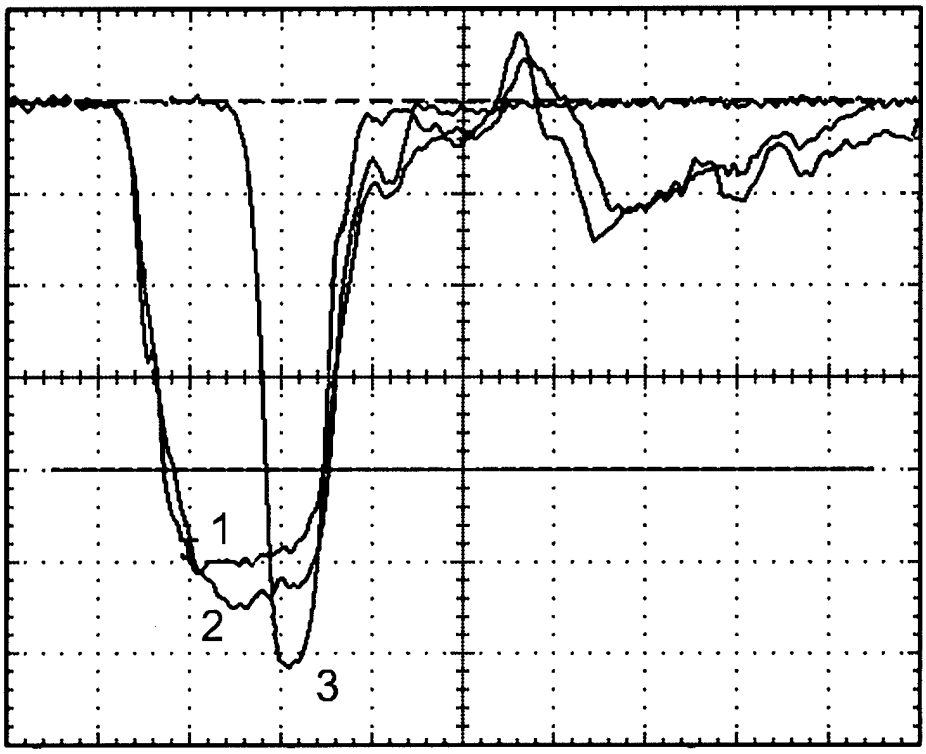


Fig.1.



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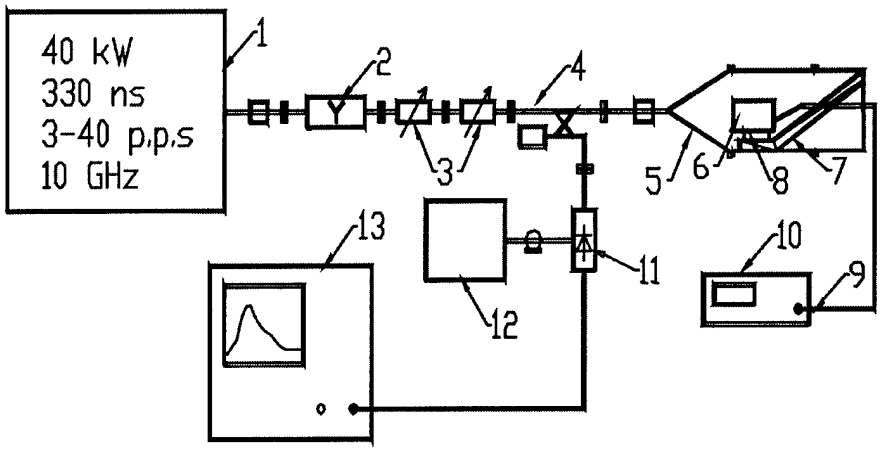

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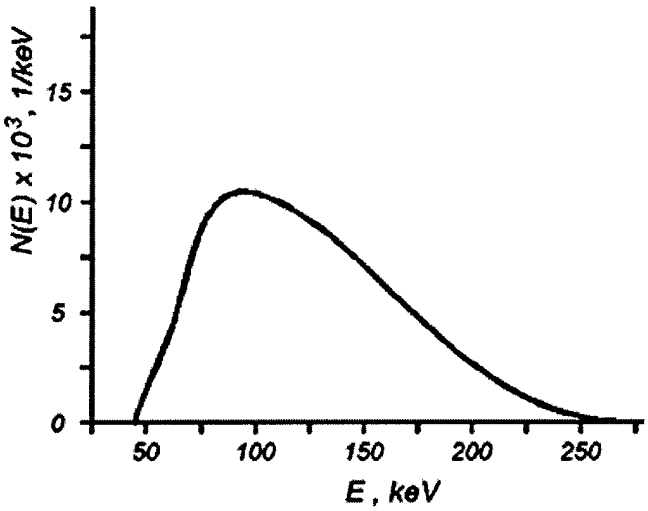
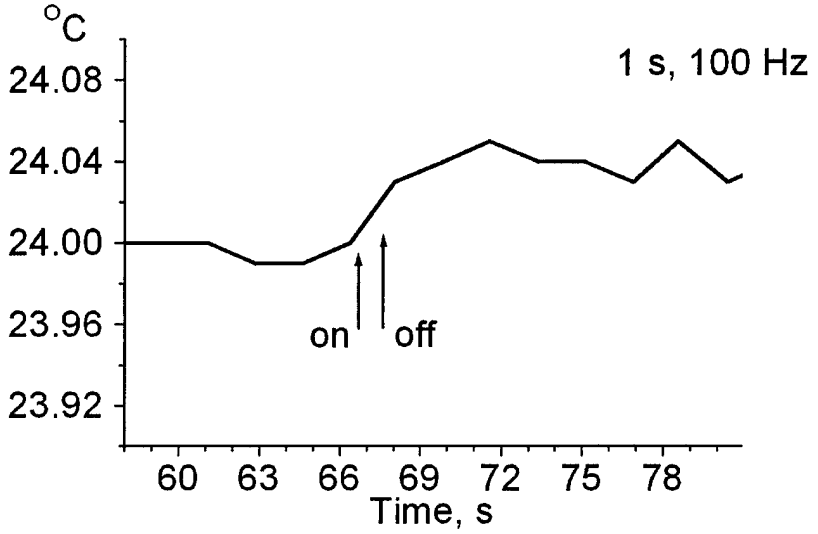


Fig. 4.



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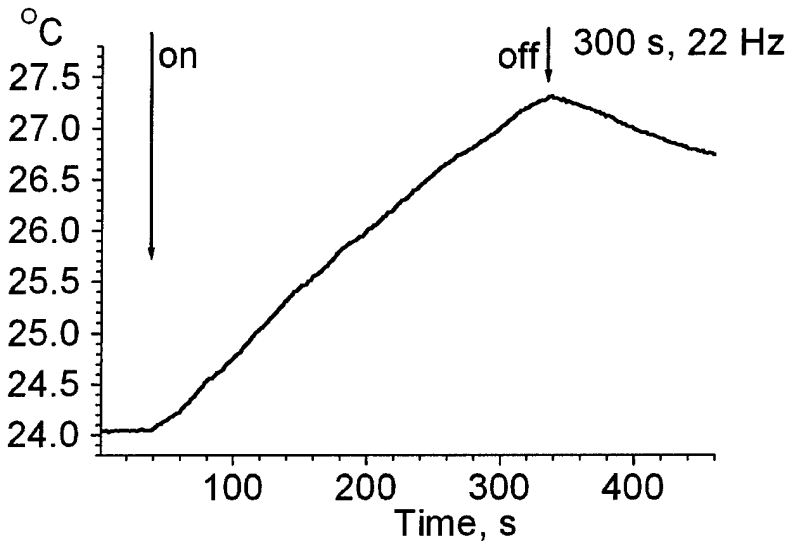

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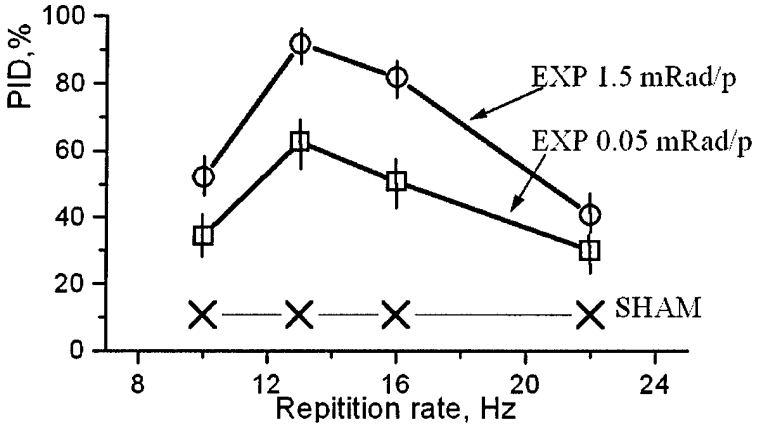

Fig. 7

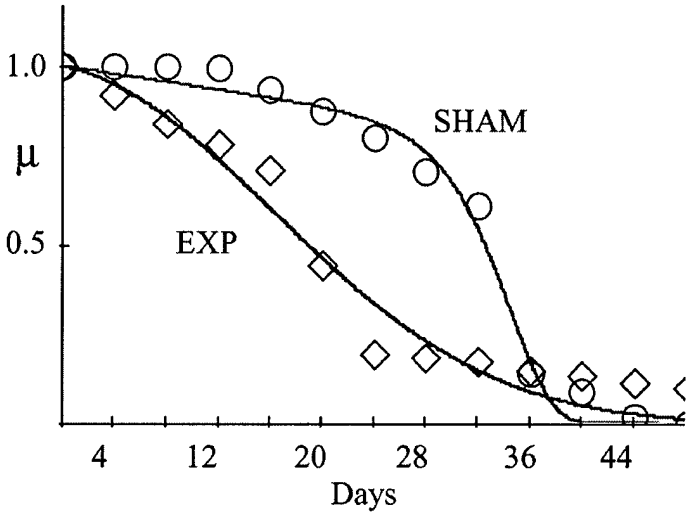

Fig. 8