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FINAL REPORT

STUDY OF THE INFLUENCE OF CELL-PHONE RADIATION ON LIVING ORGANISMS AND DAR UNIT PROTECTIVE PROPERTIES

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Moscow, 2002

According to the order of stock company DAR researchers of the Biological Faculty of the M. V. Lomonosov Moscow State University conducted investigations aimed at revealing of the negative effect of electromagnetic radiation (EMR) of cell-phones on biological objects and ascertainment of characteristics of DAR protective unit designed for reduction of the indicated negative effect.

Earlier 5 preliminary series of experiments had been conducted on the models of ontogenesis (more than 500 embryos of frog and loach) and reparation of damages (more than 200 individuals of flatworms - planarians). The present report covers the results of investigations carried out on DAR protective unit in 2002.

In the process of development and vital activity each organism is inevitably exposed to the effect of exogenous and endogenous factors. The result of such impact may be pronounced in the disturbance or modification of the processes of functioning of the whole organism or some groups of organs or tissues. It is known that there are so-called "critical periods" in the process of early development of organisms when their sensitivity to the effect of different factors sharply increases as the determination of ways of future development is going on and even the slightest unfavorable impact can disturb the normal course of further development. Such disturbance manifests itself rather quickly by such parameters as retardation in the stages of embryonic development, disturbance of synchronism of development, increase of the percentage of lost embryos, occurrence of deformity, and others. Disturbances on early stages of development later result in significant deviations affecting viability of an individual.

Besides multiple regulation processes are to go on through the life of an individual aimed at returning of an organism to the state of physiological norm (or to the maximum close to it state) if some factors of exogenous or endogenous character disturb its stability. Regulation processes work at different levels: molecular, cellular, organism, and so on. An example of regulation is regeneration process. Regeneration processes intrinsically repeat individual development and in this case practically all basic processes determining formation of any organ (from proliferation of cells, their posterior migration, differentiation, growth, etc.) can be observed. Besides it should be indicated that in the process of regeneration many genetic programs working at early developmental stages of organisms switch on.

Capability to protect these processes from potentially unfavorable external technogenous factors (such as electromagnetic radiation of different sources, including cell-phones) undoubtedly has vital importance for everybody who is exposed to such impact.

In the present research we used staged approach to assess protective properties of devices designed to decrease the effect of cell-phones radiation. We studied their protective effect on the processes of reparation of organisms' disturbances (model of regeneration of flatworms'- planarians'- bodies cut into halves) at the first stage, and on the processes of ontogenetic development of organisms (on the models of development of fishes and amphibians from eggs) at the second stage.

Such approach permits revealing both of reactions of biological objects significant for the formation and resistance of an organism on the impact of cell-

phones EMR and display of protective properties of DAR units concerning cell-phones radiation.

Experiments were conducted through winter and spring periods 'under artificial lighting in room temperature. All biological objects in each series stayed under the same conditions and were subjected to the same manipulation procedures. Irradiation was conducted in the evening (from 6 to 8 p.m.) and diagnostics and testing was made in daytime (2-5 p.m.).

Parameters of the impact were close in all experiments. In all cases we used cell-phones Motorola M3788 as sources of EMR. Cell-phone's antenna was located at a distance of 5 cm from investigated object (Fig. 1).

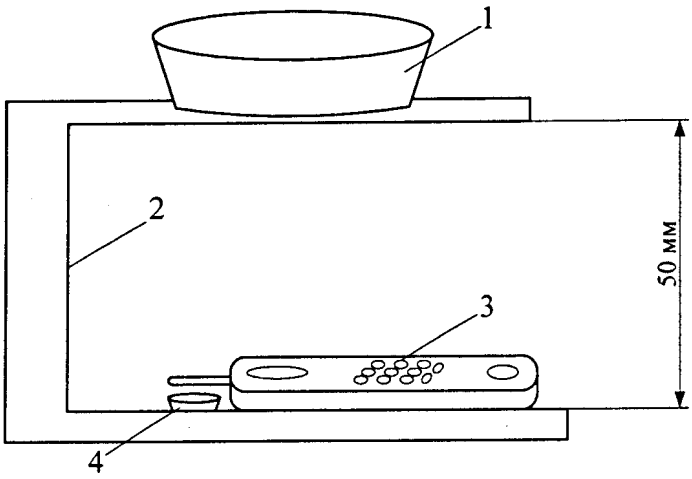

Fig. 1. Scheme of experimental installation

1. petri dish with experimental object
2. stand (height - 5 cm)
3. cell-phone
4. protective device

Modes of operation and radiation parameters of the cell-phone

Metrology of cell-phones' radiation was carried out by the specialists of the RF State Scientific Center, who used various metrological equipment.

To solve the task of determination of radiation power levels in the near zone a small-sized sonde was designed. It was calibrated with the help of standard instrumentation.

During the experiment the cell-phone Motorola M3788 was functioning in call mode within up to 2 minutes. During the first 5seconds after each actuation of the call mode, average value of the energy flow density in the center of the

experimental chamber was 120 mcW/cm2. Average value of the energy flux density then reduced up to 48-50 mcW/cm2. Irregularity of power levels of the cell phone radiation in the experiment did not exceed 3 dB. Sound indication of keystrokes was switched off.

Investigations on reparation model

Regeneration is a reparative morphogenesis which always has a multilevel character and varies in mechanisms depending on specifics, degree and localization of damages and also on the stage of individual development and complexity of organization of an individual or a colony (Korotkova, 1997).

Regeneration can be referred to the large group of regulation phenomena. It comprises all biological processes the entity of which is in returning (or bringing nearer) of an organism to the state of physiological norm if some factors drive it out of this state.

The regeneration process in planarians discussed in this work can be referred to reparative regeneration (Svetlov, 1978) by means of epimorphosis (Morgan, 1901). If planarian is cut into several pieces in arbitrary manner each fragment develops in a new individual. Several cases of reparation of these animals from 1/300 part to whole organism were described. Such extraordinary ability for reparative regeneration has made planarian a classical object for investigations of this phenomenon. Epimorphic regeneration is reparation of the lost part of the whole organism by means of forming of regeneration blastema by proliferation of cells on the amputation surface and cell migration from old tissues with subsequent differentiation, morphogenesis and growth resulting in formation of a copy of the lost structure. It has been recently shown that during regeneration process many genetic programs working at early developmental stages are actuated. Regeneration process can be used as a convenient model for investigation of the influence of exogenous factors on development.

The study was conducted on the model of reparation processes in the representatives of the type Plathelmintes (flatworms), class Turbellaria (turbellarian worms). Turbellarians are free-living worms. Many species of this class inhabit seas and oceans, the others occur in fresh-water reservoirs. They can be easily found in various water-bodies where they usually crawl on the bottom or over aquatic plants.

Planarians used in experiments represent species with exceptionally asexual reproduction based on regeneration. In this case an individual is divided by transverse constrictions into several filial individuals (Sharova, 1999).

The ability to: recover continuity after the injury or loss of a tissue (regeneration) is a fundamental property of living organisms. If you cut planarian into halves, lengthways or breadthways, very soon a new animal will regenerate from each part. The repairing fragments have a distinct polarization so that head is forming from their fore part and tail - from the hind one. As soon as the wound heals a structure having the form of cupola begins to grow in the cut place. This structure

called blastema or regeneration bud (regenerate) by its first investigators appears during the first three days after damaging of the worm's body. Then it starts growing and after necessary cell transformations forms those body parts that need repair (Natali, 1947).

In our experiment each planarian was dissected across strictly behind cephalic lobes, which present a precise marker for dissection of the cephalic part of planarian's body.

Experimental objects were irradiated with EMR of the cell phone in the call mode for 60 minutes per day during the first 3 days after the dissection of planarians' body. The whole time of irradiation was divided into several sessions so that duration of one call was 90 seconds.

To register dynamics of regeneration process we measured the surface area of regenerate three days after the beginning of the experiment. This time was chosen as during the first three days after the amputation of cephalic part firstly the process of tightening of the wound with the help of surrounding it muscle cells begins, then epithelial cells begin to move over the wound surface and in two-three days epithelium covers it all. Under the epithelium the processes of cleaning of the wound begin (loss of cells due to their destruction and histolysis) and then the processes of dedifferentiation of old cells and proliferation of parenchymatous cells start, which results by the end of the third day in the formation of regeneration tubercle (blastema) that can be registered. Through the fourth-fifth days processes of cell proliferation go on actively, and due to it the size of blastema increases. Besides the processes of cell differentiation in blastema start. Thus irradiating the objects of experiment with cell-phone EMR during the first three days we affect the whole complex of processes occurring in animal's organism in course of regeneration and any retardation in formation of blastema in this period is indicative of some disturbances of these processes. Such disturbances can be hardly revealed on late stages of regeneration processes as by this period the first two stages of regeneration process (healing of the wound and storing of regeneration material in regeneration tubercle - blastema) are completed and processes of growth start.

During winter period binocular and coordinate scale were used for measurements. Relative dimensions of regenerate (relation of blastema size to body size) were determined. The use of non-dimensional magnitudes made possible clear comparison of results of different series though the size of blastema and this magnitude were correlated with high significance, which allowed assessment of the process dynamics using both ones.

During spring period registration of the dynamics of regeneration process was carried out by means of computer-aided measurement of regenerate's surface area. Processing of the material was conducted on the base of computer programs "Fly Video 98" and "Planar". Student criterion, Fisher exact method and non-parametric Mann - Whitney criteria were applied for statistical processing of the results.

In each experiment study of 3 groups was conducted:

- control group (planarians not affected by irradiation);
- test group of planarians exposed to irradiation of cell-phone;
- test group of planarians exposed to irradiation of cell-phone under the protection of DAR unit.

In each group 10 dissected animals were used. Planarians were placed in plastic (radio parent) petri dishes filled with 20 ml of water. The water had been preliminary precipitated for 3-4 days at room temperature.

On the whole 3 series of experiments were conducted (February, April, May) with about one-month inter-series interval.

Results of experiments

Our data indicate that everyday effect of the cell-phone under experimental conditions in 3 days resulted in 1.5-time retard in the speed of regeneration process in the test group compared to the control group (which had not been affected by the cell-phone radiation (Table 1). Radiation of cell-phones with indicated above parameters hampered regeneration in planarians in spite of a season and in winter season even resulted in their 100% loss.

Application of the protecting unit DAR significantly ($p < 0.05$) reduced the mortality of worms (60% survived) and led to the compensation of the reparation process delay.

Table 1

Influence of cell-phone radiation on regeneration ability of planarians and protective effect of DAR unit

Ratio of regenerate size to body size, %				
Series		Control group	Cell-phone	Cell-Phone + DAR protective unit
1	Mean Std. err.	5.68 0.006	-	5.5 0.026
Area of blastema (pixels)				
2	Mean Std. err.	447.1 41.61	**336.6** **28.92**	530.9 70.34
Ratio of the regenerate size to body size				
3	Mean Std. err.	3.652 0.35	**2.466** **0.28**	3.563 0.275

Note: Values are boldfaced, which differ significantly ($p<0.05$) from the control ones and from the variant with the use of DAR unit.

Investigations on ontogenetic models

The objects of this study were fertilized eggs and larvas of secondary-aquatic anural amphibian - clawed frog *(Xenopus laevis* D.) and teleost fish -loach *(Misgurnus fossilis* L.).

The merits of clawed frog *(Xenopus laevis* D.) as an experimental object are the opportunity to get sexual products in considerable amounts in concrete time throughout the year and fulfill fertilization under laboratory conditions, considerable number of eggs in one set, rather high speed of embryonic and larval development and possibility to carry out accurate assessment of embryo's state at concrete developmental stages, besides any deviation from the norm can be easily revealed at visual observation. For precise assessment of stages of embryonic and larval development we used the table of normal development for the clawed frog (Niewkoop, Faber, 1956). Artificial spawning was elicited in laboratory conditions with injections of chorigonic gonadotropin: males were injected with 100-200 IU and females - with 300-400 IU (Rudneva; Gurdon, Woodland, 1975).

For the experiments on the embryos of anural amphibians we selected individuals at the definite stage of embryonic development and of the same size. As it was expedient to choose as initial stage one of the critical stages of embryonic development we selected stage of neurula (start of the formation of nervous system).

The teleost fish - loach *(Misgurnus fossilis* L.) is one of the most popular objects for the modern studies in developmental biology. The merits of this object are the following: well elaborated method of obtaining of sexual products beyond the dates of natural breeding season; short periods of embryogenesis (50-52 hours at 21 ° C) and pre-larval development (up to 10 days); easiness of keeping of embryos and pre-larvas in laboratory conditions; and possibility to make precise assessment of embryos' state on definite stages of embryonic development at visual observation. For accurate assessment of the stages of embryonic and pre-larval development we used the table of normal development for *Misgurnus fossilis L.* (Kostomarova, 1975).

Stimulation of ovulation was carried out by injection of hypophysial hormones (depending on individual size and season of the year, from 100 to 300 IU to a female) by method of A. A. Neiphakh (1959). After the injection a female was placed in crystallizer with settled water. The ovulation in females occurred in 40 - 44 hours at 19°-20° C. Ripe eggs were collected by squeezing them out from a female's belly to a dry petri dish. Insemination was carried out by newly prepared homogenate of testicles (a testicle was taken from decapitated male, then it was cut down to small parts with scissors and then settled water was added), which was poured out to the petri dish with eggs. After smooth rocking for 1-2 minutes the dish was filled with settled water, and eggs were left for further development.

For this series of experiments we used the loach embryos at the stage of gastrulation.

Methods of investigation

To study the possible effect of cell-phone and protective devices on the processes of early development in anural amphibians and teleost fishes we took their eggs passing through particular developmental stages: at the stage of neurula in anural amphibians (clawed frog) and at the stage of gastrula in teleost fishes (loach). 50 eggs were placed in each plastic petri dish filled with settled tap water. We accomplished everyday control of the state of tested groups of embryos and lost individuals were taken away from petri dishes. In 3 days the stages of development were determined in alive embryos in control group and test groups. For statistic processing of the data we applied the computer program "Stadia".

The irradiation procedure was the same as in the experiments on planarians.

Results

Study of the possible influence of cell phone on early development of secondary aquatic anural amphibian - clawed frog and teleost fish - loach showed that immediately after the impact on the embryos no alterations in their development were observed in comparison with the ones from the control group.

After 3 days of every-day sessions of cell-phone effect on these biological objects significant difference was revealed between test and control groups of embryos with the tendency of retardation in development in test groups of both clawed frog and loach (Table 2). No losses of embryos were observed in the test group as well as in the control group.

The results of the study of the effect of cell-phone radiation on the processes of early development in loach are indicative of significant decrease in the speed of development in individuals affected with cell-phone (retardation by 1 stage).

Analogous studies of the processes of early development in clawed frog also demonstrate significant slowing of development at the influence of cellphone (retardation by 2 stages). At the same time even retardation by 1 stage can be an evidence of significant distinction in the state of concrete object. For example (Fig.2) stage 34 of the clawed frog is the stage when cardiac contractions only begin to appear and embryo still stay in coats and stage 36 determines the start of hatching out and turn to self-supporting living (beyond coats).

The use of DAR protective device in both cases reduced significantly the influence of cell-phone declining the effect of developmental retardation.

Table 2

Effect of cell-phone and DAR protective unit on early development of the clawed frog *(Xenopus laevis* D.) and loach *(Misgurnus fossilis L.)* Stage of development 3 days after the affect

Experimental conditions	Stage of development *Xenopus laevis D.*	Stage of development *Misgurnus fossilis L.*
Control group	36	36
Cell-phone	34	35
Cell-phone + DAR protective unit	38	36

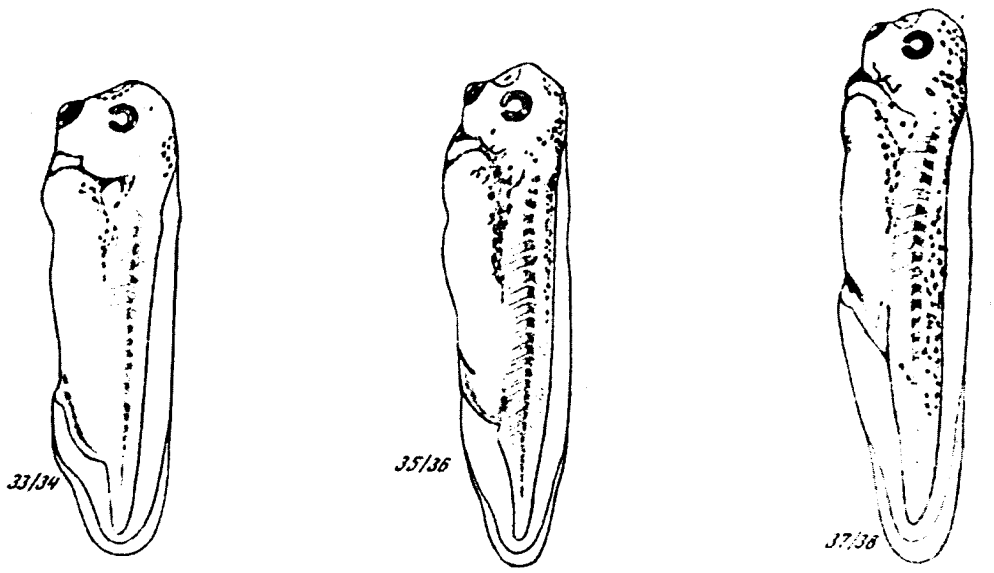

Fig. 2. Stages of development of the clawed frog *(Xenopus laevis D.)*

Conclusion

Results of experiments indicate, that impact of the cell-phone with indicated above parameters has negative influence on the processes of early embryonic development in two groups of vertebrates and on regeneration processes in flatworms. It is pronounced in retardation of embryonic development as well as of the process of regeneration blastema formation in the objects, exposed to the effect of cell-phone, compared to the ones, which developed under standard conditions.

The same result of the cell-phone impact on different biological objects and different models can be explained by the work of actually analogous mechanisms (cell proliferation, their further differentiation, cell movements, growth, work of the same genetic programs, and so on) in regeneration process and in the process of early development. Thus effect of cell-phone may disturb some of indicated mechanisms despite the model and type of biological object.

It should be emphasized that such effect of cell-phone radiation is analogous in the degree of its expressiveness to the effect of external unfavorable factor of medium intensity (for example, such factors as decrease of optimal temperature of development by 25%, or 1.5-time increase of background radiation, or exceeding by 30 % over the maximum permissible doze of heavy metals, 4-times reduction of oxygen content in the air pumped through aquarium, compared to atmospheric air (up to 5 %). Thus we observed an effect analogous to essential worsening of living conditions of biological objects.

Application of DAR protective unit together with a cell-phone led to compensation of radiation impact of the latter in all studied biological objects.