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The effect of cosmic rays on biological systems – an investigation during GLE events

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Abstract. In this study, first direct and circumstantial evidences of the effects of cosmic rays (CR) on biological systems are presented. A direct evidence of biological effects of CR is demonstrated in experiments with three cellular lines growing in culture during three events of Ground Level Enhancement (GLEs) in the neutron count rate detected by ground-based neutron monitor in October 1989. Various phenomena associated with DNA lesion on the cellular level demonstrate coherent dynamics of radiation effects in all cellular lines coincident with the time of arrival of high-energy solar particles to the near-Earth space and with the main peak in GLE. These results were obtained in the course of six separate experiments, with partial overlapping of the time of previous and subsequent experiments, which started and finished in the quiet period of solar activity (SA). A significant difference between the values of multinuclear cells in all cellular lines in the quiet period and during GLE events indicates that the cause of radiation effects in the cell cultures is an exposure of cells to the secondary solar CR near the Earth's surface. The circumstantial evidence was obtained by statistical analysis of cases of congenital malformations (CM) at two sites in the Murmansk region. The number of cases of all classes of CM reveals a significant correlation with the number of GLE events. The number of cases of CM with pronounced chromosomal abnormalities clearly correlates with the GLE events that occurred a year before the birth of a child. We have found a significant correlation between modulations of the water properties and daily background variations of CR intensity. We believe that the effects of CR on biological systems can be also mediated by fluctuations in water properties, considered as one of possible mechanisms controlling the effects of CRs on biological systems.

1 Introduction

Solar-terrestrial relations and influence of cosmophysical agents associated with solar activity (SA) on living beings have been studied by many scientists, starting with St. Arrhenius (Arrhenius, 1900). Chizhevsky (1995) was the first to extend the previous findings and to lay the foundation for geliobiology. The main research in heliobiology aimed at finding correlations between SA, variations of geomagnetic field (GMF) and functional state of living systems, including the human organism (Dubrov, 1974; Feinleib et al., 1975; Szczeklik et al., 1983; Belisheva et al., 1994a; Vladimirsky and Temuryants, 2000; Chernouss et al., 2001; Mendoza and Sánchez de la Peña, 2010).

At the same time, biological effects of secondary cosmic rays (CR) near the Earth's surface, or terrestrial CR (Ziegler, 1996) associated with variations in GMF and SA, was doubtful because of their low intensity and small oscillations of the nucleon CR component near the Earth's surface. For reference, the global variation of CR with cutoff is about factor 2 from the equator to the pole at sea level, and 3 at the highest inhabited altitudes. Solar modulation is even smaller, i.e. $\sim 25\%$ decrease from maximum to minimum recorded monthly-averaged rates at polar locations near sea level and $\sim 7\%$ at the equator, and 30% to 12% for polar and equatorial sites at high elevations (JEDEC Standard, 2006). The amplitude of the variable component of the total GMF increases almost by an order of magnitude from the equator to the poles and by more than two orders of magnitude during global geomagnetic disturbances in the area of the polar oval belt (high latitudes) (Yanovsky, 1978). Therefore, the active dose assessment and fundamental effects of exposure of biological systems to CR are studied on spacecraft in low earth orbits and on a board of aircrew (Akoev et al.,

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1989; Akoev and Yurov, 1984; Beck et al., 1996; Reitz, 2001; Singleterry et al., 2001; Belli et al., 2002; Autischer et al., 2007). At the same time, there are many researchers who study the effects of exposure of human organism to combined cosmophysical agents (space weather), including the variations of CR intensity (Dorman et al., 2001; Stoupel et al., 2007; Mavromichalaki et al., 2008; Dimitrova, 2009; Papailiou et al., 2010). Various indicators (infarct myocardial, variability of heart rate, brain strokes, car and train accidents, etc.) only suggest that the human organism responds to any stimulus (Mavromichalaki et al., 2008), deflecting it from a current state (according to Weber-Fechner Low). To explain such phenomena by the effect of certain cosmophysical agents, we need to know the targets and mechanisms of certain agent effect on the targets. Thus, cosmic rays are only "an indicator of space weather influence" on the whole organism whose parameters (variability of heart rate, arterial blood pressure, state of the brain, etc.) reflect an integral response of the whole organism to the combined cosmophysical effects, and do not provide information about the nature of effecting agents and the targets for their impact.

At present, only for ionizing radiation the targets and mechanism of its effects on the targets are recognized (Akoev et al., 1989; Akoev and Yurov, 1984; Singleterry et al., 2001). Only genomic changes are the major indicators of the exposure of cells to ionizing radiation. Ionizing radiation produces a wide range of DNA lesions in cells, such as base damage, sugar damage, single strand breaks (SSB), double strand break (DSB), DNA-DNA and DNA-protein cross links. With these numerous types of lesions, emphasis is currently placed on DNA DSB as the most important for radiobiological effects, including a chromosome aberration, cell killing (apoptosis) and transformation (Singleterry et al., 2001) (the first step to cancer). At the level of the whole organism, impaired DNA structure can lead to genomic instability, chromosome aberrations and increased risk of cancer and congenital malformations with children. Some findings provide an indirect evidence for the effects of CR on human organism near the Earth's surface (Halpern et al., 1995; Stoupel et al., 2005; Juckett, 2007). Juckett (2007) found correlation between the latitudinal dependences of cancer incidence in forty two countries and the intensity of the background CR. In addition, excellent investigations were carried out by researches in Israel (Stoupel et al., 2005). They found a strong trend towards a relationship between CR intensity and the incidence of Down syndrome.

It should be noted that for study of CR effects on biological systems, solar proton events (SPE) associated with Ground Level Enhancement (GLE) of secondary CR particles detected by ground based neutron monitors are of a particular interest. Registration and study of SPE started in the 1940s (Shea and Smart, 1995). The largest SPE occurred on 23 February 1956 with enhancements over 4000% recoded at ground-based stations. On 20 January 2005, the second in intensity SPE occurred, with a 400% enhancement registered. Starting from 1942, the year of 1989 is recognized as one of GLE event maximum. An extraordinarily large number of sunspots and solar flares, energetic particle events in the near-Earth space environment, and ground level neutron enhancements were detected in this year (Reeves et al., 1992). A drastic increase in the intensity of secondary solar CR near the Earth's surface gave a unique opportunity to study the effects of CR on biological systems.

In this study, the results of experiments performed on cellular cultures before, during, and after GLE events in October 1989 are presented. They provide the first direct evidence of biological effects of CR near the Earth's surface. In addition, preliminary results of statistical analysis of the link between the rates of congenital malformations and GLE events are also demonstrated. We consider the fluctuations in water properties under exposure to background variation of CR as one of the possible mechanisms mediating the effect of CR on biological systems.

2 Materials and methods

Cell cultures. The investigation has been carried out at high latitudes (66.3° N and 33.7° E) at the geophysical recording site (Island Srednyi, White Sea) of the Physical Research Institute of the St. Petersburg University and at the Kola Science Centre (KSC) of the Russian Academy of Sciences (RAS) in Apatity. Six separate biological experiments were conducted on 15–31 October 1989 on cellular lines grown in cultures: a mouse hypodermic connective tissue (L-line), a Chinese hamster ovary (CHO-line), and an epithelial cell line derived from Fat Head Minnow Pimephales promelas (FHM-line). Cells were maintained in 199 and Eagle medium (1:1), supplemented with 10% of cattle serum and antibiotics, 1 mmol glutamine at 37°C (Belisheva et al., 1994b). At the beginning of each experiment, cellular monolayers from the culture flasks were dispersed by a mixture of Trypsin–EDTA (1:1) and a single cell suspension in culture medium were inoculated into the antibiotic flask in quantity of 50 000 cells, where cells adhered to the surface of the cover glasses. The number of antibiotic flasks for each cell line in each separate experiment was about 40. During the period of cell cultivation on the cover glasses, 3–5 samples of antibiotic flasks for each cell lines, in each experiment, every 3, 6, and 12 h were selected for fixation of cells. Before cell fixation, 1 μKu/ml of 3H-thymidine was added for 30 min for far visualization of nuclei with DNA synthesizing activity. The cover glasses with the cells were washed by physiological solution and were fixed by a mixture of methanol: acetic acid (3:1). For visualization of DNA synthesizing sites in the cells, the glasses with adhering cells were marked by the 3H-thymidine, were covered by a photo-emulsion (Ilford), were kept for three months in the dark, were developed, were stained with hematoxilin-eosin, and were then made permanent preparations for far analysis.

Morphofunctional analysis. In order to estimate the morphological changes in cellular lines, permanent preparations were analyzed by epi-fluorescence microscope Zeiss Axioskop 2, coupled with image device and software for image analysis (Media Cybernetics, Inc.). Cells with single nuclei, with gigantic nuclear, with micro nuclear, and with multinuclear cells (MNC) were counted with the microscope by using 10–20 fields of view. The total number of counted cells was about 1000 on each cover glass for each experiment. The morphofunctional dynamics were estimated by index of MNC (percentage of MNC in the cell population normalized against the mean MNC during the quiet period (15–18 October 1989). For analysis of morphofunctional dynamics, the indices of MNC in six separated experiments were averaged on coinciding time serious points for each cellular line.

Case ascertainment and data collection. Cases of congenital malformations among births in Kandalaksha and Monchegorsk sites (Murmansk region) were collected by Dr. L. V. Talykova (North-West Scientific Centre of Occupational Health and Social Health of the Russian Consumer Surveillance. Research Department for Occupational Health, Kirovsk, Russia). Cases were defined on the basis of International Classification of Diseases-10 (ICD-10): chapter XVII, block Q00–Q99 (Pregnancy, childbirth and the puerperium) and class Q90–Q99) (congenital malformations, deformations and chromosomal abnormalities) through state vital records of the pregnant women and newborns, diagnostic indices, autopsy records. The number of live births, fetal deaths, congenital malformations for each year between 1989–1998 (Kandakaksha) and betwen 1987–2005 (Monchegorsk) in hospitals were determined (per 1 000 relevant population).

Geophysical data. Ground-based neutron monitor data in the corrected and uncorrected on atmospheric pressure were provided by the station of neutron monitor of the Polar Geophysical Institute (PGI), KSC RAS in Apatity (67.34° N, 33.24° E). The data on solar activity and particle fluxes in the near-Earth environment during considered time periods were taken from the WCD (Solar-Geophysical Data Prompt Reports, No. 543 – Part I 1989). GOES-6 satellite data on nuclear-active particles, which are able to produce secondary CR and to achieve the Earth's surface at the site of the experiments (magnetic cutoff rigidity of 1 GV, corresponding to the proton energy > 430 MeV), were selected. We also used the data on protons with energies $> 850, 640-850, 480-640$ and > 100 MeV, as well as on $\alpha-$particles in the energy ranges of $> 850, 630-850$ and $330-500$ MeV for a comparison with dynamics of MNC-indices.

Statistical analysis. All data for cellular lines, cases of congenital malformations, properties of water were processed, with the means, minimum and maximum values, standard deviations, and standard errors of the means were calculated. The statistical significance of the correlation between the means of MNC-indices in each cellular lines in quiet period, control before GLEs, during GLEs and after GLEs were determined by the Student's t test. The significance of the relationship between the cases of congenital malformations and the rate of occurrence of the GLE events was estimated from correlation matrices. A p value of 0.05 or less was considered to be significant. All calculations were realized with the software Statistika: release 6.

3 Biological effects of cosmic rays

The CRs near the Earth's surface are secondary particles of low intensity with high LET. Therefore, the assessment of biological effects of CR is a complicated problem for several reasons.

One difficulty is associated with detection of real nuclear and energetic spectrum of secondary particles near the Earth's surface. "Most experiments, which measured the CR flux of particles with the nuclear force (hadrons), did not separate the flux into neutron, proton, and pion components. There is a general agreement that the flux of particles is more than 97% neutrons at sea level. There were no quantitative estimates of the total sea-level cosmic ray nucleon flux. In performing measurements in the high-energy range, there is an uncertainty of $2x$ because of unknown angular distribution of the neutron flux at sea level. The shapes of the flux spectra (flux vs. energy) of various particles are known to better than $2\sim$. There are problems with the absolute normalization of the neutron curves to obtain absolute fluxes" (Ziegler, 1996).

The second difficulty is a consequence of the first one: the most biologically effective part of the secondary particle spectrum is not measured because of the lack of adequate equipment. The maximum of biological effectiveness for neutrons falls in the energy range $0.1-0.4$ MeV (fission neutrons). As the neutron energy increases, the LET average values of secondary particles decreases and so do their biological effects (Cowan, 1967).

The third difficulty is associated with a bystander effect. It has always been agreed that the deleterious effects of ionizing radiation such as mutation and carcinogenesis are attributable mainly to the direct damage to DNA. This is based on the dogma that the DNA of the nucleus is the main target for radiation-induced genotoxicity. The modern radiobiological researches demonstrate that bystander mutagenesis in mammalian cells is induced by a single alpha particle through the nucleus. The frequencies of induced mutations and chromosomal changes in populations where some known fractions of nuclei were hit are consistent with non-hit cells contributing significantly to the response. In fact, irradiation of 10% of a confluent mammalian cell population with a single particle per cell results in a mutant yield similar to that observed when all of the cells in the population are irradiated (Zhou et al., 2000, 2001).

Thus, only the biological indicators of radiation effects, such as DNA lesions, can be a direct evidence of biological effectiveness of CR.

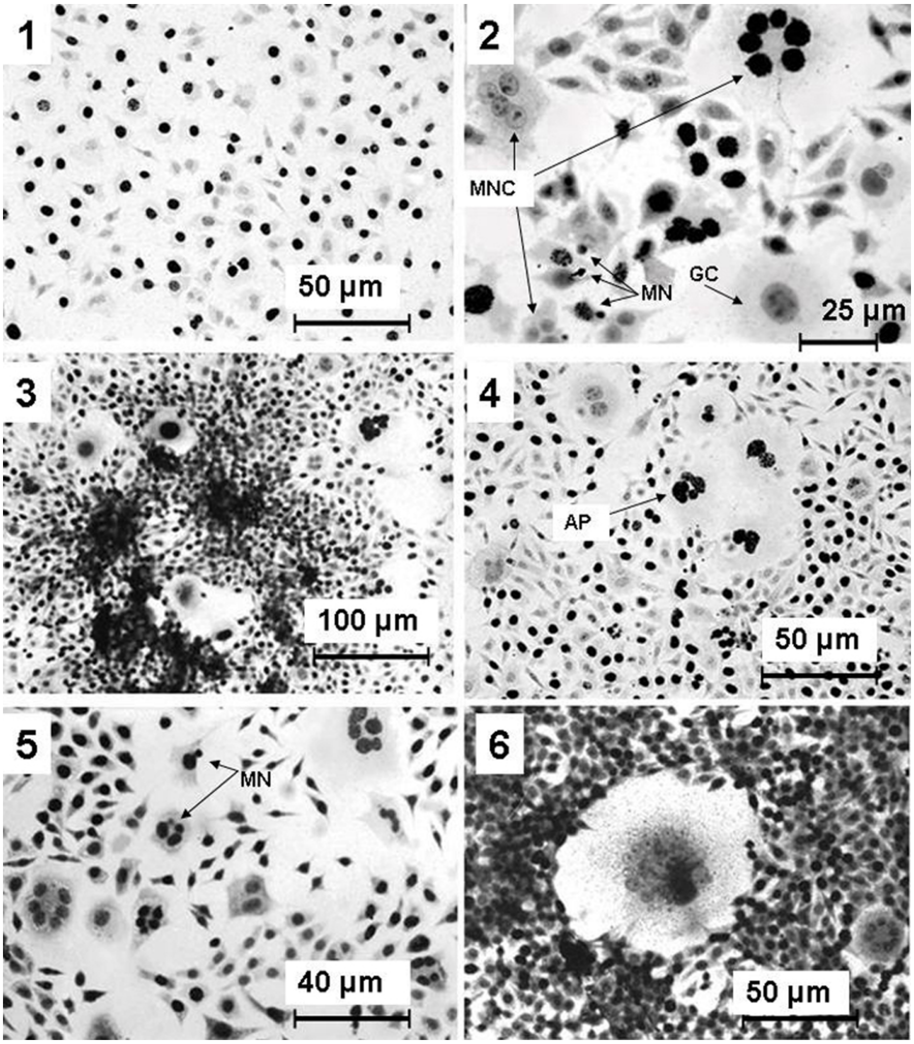

Fig. 1. Morphofunctional state of cell culture during quiet period and GLE's events (illustrated with L-line sample). The black color corresponds to the DNA-synthesizing nuclei marked by 3H-thymidine; incorporation of the 3H-thymidine into the cell nuclei is the characteristics of cell functional activity. **(1)** Cellular monolayer in the quiet period; **(2)** cellular monolayer during GLE 43, October 19, 1989. The arrows show the multinuclear cells (MNC), gigantic cells (GC), micronuclei (MN); **(3–4)** local clusters of nuclear disorders: MNC, GC; apoptosis (AP); **(5)** cellular monolayer during GLE 45, abundance of MNC and separate MN are seen in the figure; **(6)** apoptosis of gigantic nucleus after GLE 45 (in the centre of the figure).

3.1 Morphological changes in cell cultures

We have carried out experiments to investigate the effects of exposure of geomagnetic field (GMF) variations on the morphofunctional state of three different cell cultures at high latitudes (66.3° N and 33.7° E), where the GMF variations are most intense. Fortunately, the period of our experiments coincided with great solar energetic particle events (October 1989), which were associated with three events of ground level enhancements (GLEs) in the neutron count rate detected by a ground-based neutron monitor at the Apatity site.

Extraordinary phenomena as compared to the usual state of cell cultures (Fig. 1**(1)**) were detected in the morphofunctional state of three different cell cultures during performed experiments (Belisheva et al., 1994b; Belisheva and Popov, 1995). These phenomena manifested as (i) an abrupt increase of multinuclear cells (MNC), Fig. 1**(2–6)**; (ii) gigantic cells (GC), Fig. 1**(2–4)**; (iii) multiple disorders of cellular and nuclear substances, including the appearance of cells with apoptosis (Fig. 1**(4,6)**) and micronuclei (Fig. 1**(2,5)**); (iv) local region of clustered damages (Fig. 1**(3–6)**); (v) coherent manifestation of signatures (i)–(iv) in the three lines (Fig. 2; Table 1, Table 2). Some of damages were typical under exposure of ionizing radiation on the cells: MNC, GC, apoptosis, micronuclei (Hanson and Komar, 1985; Mariya et al., 1997; Sato et al., 2000; Belyakov et al., 2005), including a small region of clustered damages (Holley and Chatterjee, 1996; Rydberg, 1996). Moreover, clustered damages of nuclear substances were similar to the "bystander" responses, involving damage to the nearby cells that were not directly traversed by the radiation (Belyakov et al., 2005; Akoev et al., 1989).

Timing of the dynamics of the MNC-indices and the arrival of fluxes of solar energetic particles detected by the GOES-6 satellite in the Earth's orbit, and data obtained by the ground-based neutron monitor (Fig. 2), show that the increase of the intensity of ionizing radiation on the Earth's surface in association with solar proton events can be the cause

Table 1. Coefficients of correlation between fluxes of solar energetic particles, neutron count rate and MNC-indices in the L-, CHO-, FHM-lines during GLE 43: peak 1 – arrival of solar particles with hard spectrum and peak 2 (not detected by the ground-based neutron monitor) – arrival of solar particles with softer spectrum.

Increase of solar energetic particles		α−particles [MeV]	protons [MeV]				neutrons [counts/10 s]
		330 − 500	> 850	640 − 850	480 − 640	> 100	
			L-line				
GLE 43:	peak 1	0,90*	0,91*	0,91*	0,92**	0,92*	0,87*
	peak 2	0,64*	−0, 14	0,69*	0,85*	0,65*	0,64*
			CHO-line				
GLE 43:	peak 1	0,90*	0,90*	0,91*	0,91*	0,91*	0,89*
	peak 2	0,58*	−0, 03	0,52	0,57*	0,66*	0,06
			FHM-line				
GLE 43:	peak 1	0,57	0,58	0,60	0,59	0,59	0,56
	peak 2	0,54	-0,07	0,52	0,73*	0,60*	0,33

Confidence level of correlation * ≥ 95%

Table 2. Means with standard errors (SE) and significance (p) between indices of MNC in the quiet period (16–18 October), during GLEs (19–20; 22–23; 24–26 October) and after GLEs (29–31 October) in the L-, CHO-, FHM-lines.

Solar activity	October 1989	L-line Mean ± SE	p	CHO-line Mean ± SE	p	FHM-line Mean ± SE	p
Quiet period	16 − 18	$1,12 \pm 0,34$		$2,25 \pm 0,38$		$2,59 \pm 0,57$	
GLE 43	19 − 20	$7,28 \pm 0,88$	< 0.0001	$7,57 \pm 1,19$	0.0006	$6,54 \pm 0,71$	0.0004
GLE 44	22 − 23	$4,78 \pm 0,83$	0.0003	$4,50 \pm 0,33$	0.0023	$8,78 \pm 1,90$	0.0014
GLE 45	24 − 26	$4,19 \pm 0,78$	0.0001	$3,92 \pm 0,39$	0.026	$4,56 \pm 0,44$	0.0186
Quiet period	29 − 31	$1,70 \pm 0,49$	0.36	$4,90 \pm 0,71$	0.0031	$3,44 \pm 1,22$	0.48

of the identified phenomena.

Additional evidences for a cause-effect relation between solar energetic particles and the revealed phenomena are given in Table 1. The first arrival of most energetic particles, which produce a dramatic hardening of the spectrum but not much change in the flux (Reeves et al., 1992), Fig. 2, is apparent in the correlation coefficients between the MNC-indices, protons with energies > 850 MeV, and neutron count rate (Table 1). “The three GLEs observed in October 1989 correspond very well with the three main flux onsets and increases in spectrum hardness” (Reeves et al., 1992). The second arrival (Fig. 2; peak 2 in Table 1) of more plentiful, lower energy particles softens the spectrum but dramatically increases the flux (Reeves et al., 1992). This event can also be identified in the correlation coefficients between the MNC-indices and fluxes of particles with softer spectrum (Table 1), despite the fact that peak 2 is not detected by the neutron monitors (Fig. 2). Thus, we can assume that the cellular and nuclear disorders revealed in our experiments could be induced by a cascade of secondary particles, which are generated in the upper atmosphere by solar energetic particles with both hard and softer spectra and are able to achieve the Earth’s surface.

Most extraordinary phenomena associated with the first increase in the MNC indices in three cell cultures relative to the quiet period were observed during the rise time of spectral hardness that corresponded to the arrival of particles with energy > 850 MeV, GLE 43 (Fig. 1**(2)**). During this period, the values of the MNC indices were the highest: they increased with respect to the quiet period by factors of 6.5, 3.4, and 2.5 in the L-, CHO- and FHM-lines, respectively (Table 2). The next GLEs were also accompanied by an increase of the MNC indices in each cell lines by factors of 4.4, 2.0, 3.4 (GLE 44) and of 3.7, 1.7, 1.8 (GLE 45) in the L-, CHO- and FHM-lines, respectively, Table 2. When the events ceased, no significant difference was found between the MNC indices in the L- and FHM-lines relative to the quiet period (Table 2). However, in the CHO-line, high values of the MNC-indices persisted after the GLEs. Therefore, we suppose that genomic disorders induced by great solar parti-

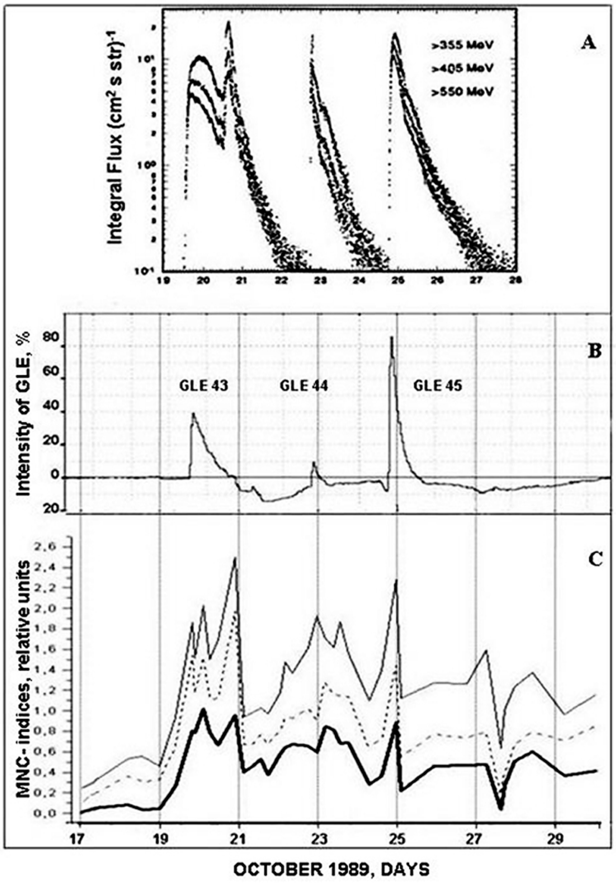

Fig. 2. Timing of the arrival of high energy solar proton integral flux as measured on the GOES-6 satellite in October 1989 (from Shea and Smart, 1995) **(A)**; intensity of ground level enhancement (GLE 43, GLE 44, GLE 45) detected by the ground-based neutron monitor at the Apatity site **(B)**; and multinuclear cell dynamics (the MNC-indices) **(C)** during great solar energetic particle events in October 1989. Top panel: integral flux of solar particles ($\mathrm{cm}^{-2}\,\mathrm{s}^{-1}\,\mathrm{str}^{-1}$); Middle panel: intensity of GLE's events, %; Bottom panel: MNC-indices in the L-line (gray solid curve), CHO-line (dashed curve), FHM-line (bold curve), relative units.

cle events can be both reversible and irreversible. In the latter case, damages of nuclear substances in the somatic cells may promote tumor processes, and their occurrence in germ cells may lead to congenital malformations.

We suggest that high energy particles, fragments and recoils, as well as the neutrons generated in the nuclear cascade (Beck et al., 1996; Reitz, 2001; Singleterry et al., 2001) during some GLEs near the Earth's surface, could be responsible for the diverse phenomena observed in the cell cultures. However, the background variations in the secondary cosmic rays produced by primary galactic particles, could also initiate similar biological phenomena, though with lower occurrence rate. A correlation between the fusion dynamics of cells growing in vitro and variations in the neutron intensity

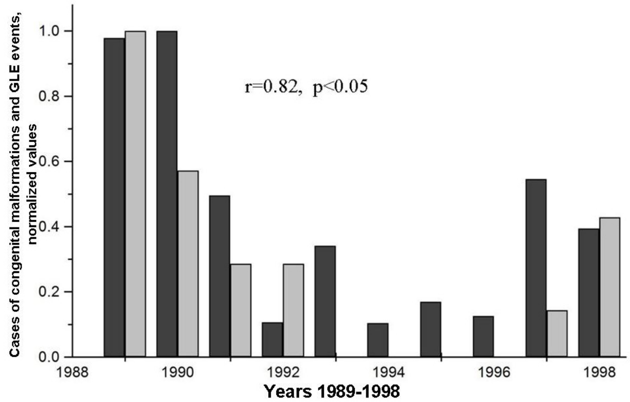

Fig. 3. Comparison of number of cases of congenital malformations at the Kandalaksha site (black column) with number of cases of GLE events (gray column) in the years from 1989 through 1998. The height of a gray columns correspond to following number of cases of GLEs: 1989 – GLEs 40–46 (7 cases); 1990 – GLE 47–50 (4 cases); 1991 – GLE 51,52 (2 cases); 1992 – GLE 53,54 (2 cases); 1997 – GLE 55 (1 case); 1998 – GLE 56,57,58 (3 cases).

near the Earth's surface supports this suggestion (Belisheva et al., 2005).

3.2 Statistical analysis of congenital malformations during GLEs

Numerous phenomena in cell cultures coupled with GLEs 43, 44, 45 manifest an increase in intensity of neutron component near the Earth's surface that induces a diverse disorder of DNA-containing material in the cellular nuclear. If such effects of GLE have a universal nature, similar DNA damages could also occur in human germ cells with far manifestation through an increase of cases of congenital malformations (CM). We have examined this hypothesis in the database on women reproductive health collected in the North-West Scientific Centre of Occupational Health and Social Health of the Russian Consumer Surveillance, Research Department for Occupational Health, Kirovsk, Murmansk region, Russia.

All cases of CM between the years of 1989 and 1998 in the Kandalaksha site and the number of cases of CM from class (Q90–99) between the years of 1987 and 2005 in the Monchegorsk site were used for preliminary analysis.

The total prevalence cases of CM (sum), average value (mean) of cases for 10 years, and range of their deviations at the Kandalaksha site are shown in the first row in Table 3. The average values of cases in the years with GLE events (1989–1992; 1997–1998) are given in the second row. The number of CM cases in the years without GLE events (1993–1996) are shown in the third row. A total of 19 GLE events occurred during the studied period. The effects of GLE events on the births with congenital malformation is seen from Table 3. In the years with GLE events, the average

Table 3. The prevalence of cases of congenital malformations at the Kandalaksha site from 1989 through 1998, including years with and without cases of GLE events.

Years	Valid N	Mean	Minimum	Maximum	Std.Dev.	Standard Error	Sum
1989 – 1998	10	17,63	4,30	41,40	13,96	4,42	176,30
1989 – 1992; 1997 – 1998	6	24,28	4,40	41,40	14,37	5,86	145,70
1993 – 1996	4	7,65	4,30	14,10	4,44	2,22	30,60

Table 4. The prevalence cases of congenital malformations with chromosomal abnormalities at the Monchegorsk site from 1987 through 2005, including years with high and low solar activity.

Years	Valid N	Mean	Minimum	Maximum	Std.Dev.	Standard Error	Sum
1987 – 2005	19	1,03	0,00	4,49	1,36	0,31	19,60
high SA	9	1,42	0,00	4,49	1,41	0,47	12,79
low SA	10	0,68	0,00	3,88	1,30	0,41	6,81

number of CM cases ($24,28 \pm 5.86$) as compared to the years without GLE ($7,65 \pm 2.22$) increased by factor ~ 3.2 ($p = 0.0584$). A clear relationship was revealed between the number of GLE events and cases of CM ($r = 0,82; p < 0,05$) (Fig. 3).

To analyze the connection of the cases of CM (Q90–Q99) determined by chromosomal abnormalities at the Monchegorsk site, with intensity of CR, the number of GLE events and their intensity (increase, %), fluxes of high energetic particles detected by GOES-6 ($\alpha-$particles with energy range $2560 - 3400$ MeV and with energy > 3400 MeV, protons with energy range $510 - 700$ MeV and with energy > 700 MeV) were considered for 1 year before the record of the births, since it was impossible to state the exact date of conception. A total 30 cases of GLE events occurred during 1987–2005. The data of descriptive statistics of number of births with congenital chromosomal abnormalities are given in Table 4. Any significant correlation between the average value of cases of CM through the years with and without GLE events was not found ($p = 0.251$). These years correspond to high and low SA, respectively (Table 4). A similar result has been obtained by Halpern et al. (1995) in their search for correlation between solar activity phases and the incidence of foetal chromosome abnormalities. Nevertheless, we revealed a significant correlation between the intensity of GLE and the births of child with chromosomal abnormalities ($r = 0.61,\ p < 0,05$).

Most disorders in the cellular nuclei discovered in experiments in October 1989 were observed during the rise time of spectral hardness that corresponded to the arrival of particles with energy > 850 MeV. Therefore, to find out whether chromosome abnormalities in germ cells associate with fluxes of high energy particles, we have estimated correlation between number of cases CM (Q90–Q99), fluxes of $\alpha-$particles and protons, cases of GLEs and their intensity (Table 5). Unfortunately, the detection of high energetic particles on the GOES-6 satellite was limited by the time span from 1986 to 1994. For this reason, we have analyzed data concerning only this time span.

It turns out that high correlation takes place between occurrence rate of cases of CM with chromosomal abnormalities, GLE events and the flux density of $\alpha-$particles within the time span between 1987–1994 (Table 5, Fig. 4). This means that GLE events associated with increase flux density of particles with hard spectrum, lead to DNA lesions in human cells, as in the case with cell cultures during GLE in October 1989.

The results of our preliminary analysis are in good agreement with the data of Stoupel et al. (2005) who found a relationship between CR intensity and the incidence of Down syndrome. These results are of principal importance for recognizing the threshold of biological effectiveness of CR: the background fluctuations in CR with maximal amplitude of $\sim 25\%$ through the minimum of SA and a drastic increase in amplitude $\sim 200\%$ during GLE events (1989) induce similar effects on chromosomal level in human cells and determinate the increase of cases of congenital malformations.

3.3 A proposed mechanism

The mechanism of exposure of CR on biological objects is insufficiently clear so far. It is assumed that ionizing radiation may act on biological targets both immediately and through products of water radiolysis (Sverdlov, 1974). Our experimental studies of the dynamics of liquid water properties conducted in the Fok Research Institute of Physics of St. Petersburg State University (Petergof, 59.53° N, 29.54° E) revealed that properties of water are modulated by background variations of CR (Vinnichenko et al., 2009) generated in the upper atmosphere by primary particles of galactic origin. In this research we applied the special experimental conditions, which allowed protection of the

Table 5. Coefficients of correlation between number of cases of congenital malformations with chromosomal abnormalities (Q90–Q99), fluxes of $\alpha-$particles ($2560-3400$ MeV, >3400 MeV), protons ($510-700$ MeV, >700 MeV), number of cases of GLE and their intensity (increase, %) during 1987–1994 years.

$1987-1994$	Q90–Q99	$\alpha-$particles $2560-3400$ MeV	$\alpha-$particles >3400 MeV	Protons $510-700$ MeV	Protons >700 MeV
$\alpha-$particles 2560-3400 MeV	0,77*	1,00	0,91*	0,42	0,24
$\alpha-$particles >3400 MeV	0,92*	0,91*	1,00	0,09	0,05
Protons $510-700$ MeV	$-0,01$	0,42	0,09	1,00	0,85*
Protons >700 MeV	$-0,05$	0,24	0,05	0,85*	1,00
GLEs (15 cases)	0,79*	0,64	0,79*	$-0,37$	$-0,51$
GLEs, %	0,89*	0,90*	0,98*	0,00	$-0,11$
Confidence level of correlation * $\geq 95\%$					

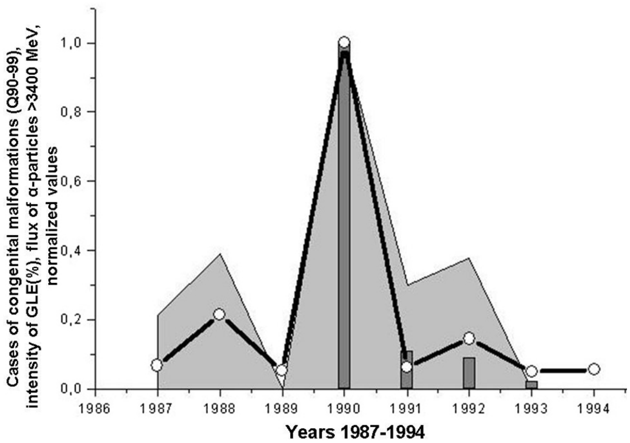

Fig. 4. Comparison of number of cases of congenital malformations (area graph, gray color), determined by chromosomal abnormalities (Q90-99), with intensity of GLE events (increase, %), (dark gray columns), and fluxes of $\alpha-$particles with energy >3400 MeV, ($\mathrm{cm}^{-2}\,\mathrm{s}^{-1}\,\mathrm{ster}^{-1}$), (bold curve).

object (ampoule with water) from the impact of external electric, electromagnetic, and thermal fields.

An aluminum Dewar vessel with a capacity of 15 l placed into a container filled with heat insulator served as a screen for protection of the sample from electric and electromagnetic fields and sharp changes of external temperature. A soldered quartz ampoule 5 cm^3 in size filled with degassed water under the pressure of its own vapors and with an inner capillary, which hosted a soldered joint of a differential thermal copper–constantan pair (emf 47.5 mkV/K), was placed into the Dewar vessel. The latter contained also the "comparison body" representing a brass cylinder with an effective heat capacity equal to that of the ampoule with water. The cylinder was equipped with the second electrically isolated soldered joint of the differential thermo pair. The material for the body was selected taking into consideration that its neutron section is substantially smaller as compared with that of the thermal and slow neutrons in water. Thus, the electromotive force of the differential thermo pair depended only on the difference between temperatures of the ampoule with water (ΔT) and the comparison body. The time constant of the heat exchange between soldered joints of the thermopair was 0.7 h. The contacts of the thermopair in the form of a screened twisted pair were connected with the electromotive force measuring system by a semiautomatic bridge P345 (class 0.001), the output signal of which was registered at the input Y of the plotter electronic potentiometer H307. The input X received a linearly variable voltage with the velocity of a device scale per 5 days. In control experiments, the bath with water was replaced by a brass or paraffin block. Inasmuch as ΔT variations in the experiment could be determined only by external sources with the required penetrating ability, we concluded that variations in the geomagnetic field and secondary components of cosmic rays could serve as such a source.

It was found that changes of temperature in the ampoule with water (ΔT) relative to the comparison body demonstrate distinct periodic daily patterns, with the peak of their maximal values near 7.4 ± 0.4 h in local time (LT), amplitude of maximum deviation 0.036°C. When soldered joints of the thermo pair were combined, the signal did not demonstrate any periodic patterns and practically coincided with the zero run of the bridge P345. We found that the local variations of the geomagnetic field are not responsible for daily variations of water properties during the experiment. We have analyzed the correlation between ΔT values and fluxes of high energetic particles measured by satellites GOES-8 (75° W) and GOES-10 (135° W) in LT and revealed that ΔT values positively correlate with variations of $\alpha-$particles with energies of $2560-3400$ MeV (GOES-10), $\alpha-$particles with energies > 3400 MeV (Fig. 5), and protons with energies > 700 MeV (GOES-8), ($p < 0.05$). Comparison of neutron count rate in the Jungfraujoch, Apatity, Moscow (IZMIRAN), and Irkutsk stations with ΔT values in LT doesn't demonstrate significant correlations. We have assumed that ΔT values and variations in neutron count rate under comparing in LT correlate with fluxes of the particles of the distinct energetic spectra, nuclear composition, and, probably, time of arrival to the site where measurements are conducted.

Thus, the experiments on the water discovered the same relations that were revealed in experiments on cell cultures and statistical material of the occurence rate of cases of CM: flux density of primary particles with a hard spectrum in the near-Earth space are correlated with the occurence rate of disoders in the cellulalar nuclei of the diverse biological objects in the Earth's surface. Therefore, we believe that change of the water properties in result of exposure of cosmic rays on the water may make some contribution to the occurence rate of the genetic disoders of the different cells. However, the occurrence rate of nuclear disorder induced by exposure of background variation on biological systems will be lower than during GLE.

The active forms of oxygen, radicals H, OH, HO_2, hydrogen peroxide generated in the result of interaction of secondary particles of CR with water molecules (radiolysis of water), could mediate the impact of CR on biological systems. Water radiolysis probably made some contribution to the "bystander" effect.

4 Conclusions

This paper presents first direct and circumstantial evidences of the effects of CR on biological systems. A direct evidence of biological effectiveness of CR has been obtained in the experiments on three cellular lines growing in culture during solar proton events (SPE) accompanied by three cases of GLEs in October 1989. Diverse phenomena associated with DNA lesion on the cellular level were found on the three cellular lines during six separate experiments, with partial overlapping of the time of successive experiments, which started and finished in the quiet period of SA. A diverse disoder of nuclear, cellular, intercellular material, as well as origin of multi-nuclear cells and cells with gigantic nuclei, micronuclei, apoptosis and other phenomena, were considered as indicators of exposure of cell cultures to ionizing component of solar CR near the Earth's surface. The dynamics of the formation of multinuclear cells was coherent in the three

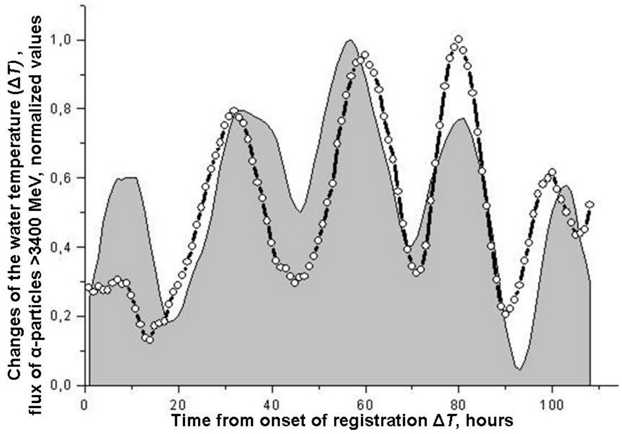

Fig. 5. Comparison of the dynamics of the changes in water temperature ΔT (area graph, gray color) in local time (onset of registration 00:00 LT) with smoothed values of variations in $\alpha-$particle fluxes with energies > 3400 MeV (bold curve) ($cm^{-2}\,s^{-1}\,ster^{-1}$) according to data from the satellite GOES-8.

cellular lines, the curves of such dynamics coincident with the profiles of solar energetic particles arriving at near Earth space, and the main peaks in the number of multinuclear cells coincident with the cases of GLE detected by a ground based neutron monitor. A total complex of synchroneous disoders of genetical material in the three cellular lines and their temporal coincidence with GLE events are direct evidence of the effects of CR on biological systems. A circumstantial evidence was obtained from a preliminary statistical analysis of CM cases at two sites in the Murmansk region. We found that the number of cases of all classes of CM reveals a significant correlation with the number of GLE events at the Kandalaksha site. The number of CM cases with pronounced chromosomal abnormalities exhibits a significant correlation with the GLE events that occurred a year before the birth of a child. We found that modulations of water properties are associated with variations in CR. We suggest that the active forms of oxygen, radicals H, OH, HO_2, hydrogen peroxide, which are generated in result of interactions between the secondary particles of CR and water molecules (radiolysis of water), could mediate the impact of CR on biological systems. In the previous section we briefly discussed why it is difficulty to estimate the real biological effectiveness of CR. We believe that the new technique based on millidosimetry would help to assess the biological effectiveness of CR.

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