Neutron Flow Exposure as a Test for Survival of *Artemia Salina* Spores

I. S. Matveeva*, A. N. Smirnov*,*, B. D. Vodennikov**, I. M. Popov**, D. S. Semenov**, M. V. Kolesnikov*, and A. V. Syroeshkin***

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 11, pp. 530-534, November, 2004
Original article submitted October 27, 2003

Live and heat-inactivated *Artemia salina* spores (samples with the same mass and filling density) were exposed to a flow of thermal neutrons from a ^{252}Cf radioactive source at an equivalent dose power of about 1 μSv/h. Irradiation led to a 4-fold acceleration of nauplius development and to modification of the element profiles of live spores. The difference between absorption/diffusion of thermal neutrons by live and dead spores was revealed.

Key Words: *neutrons; element profiles*

V. I. Vernadskii, investigating the biogeochemical differences between live and inert matter, was the first to put forward the problem about differences between them [2]. Due to new approaches it is now possible to determine kinetically different physiological states of cells, including live and dead states. Different sensitivity of dormant and active forms of cancer cells to thermal and suprathermal neutrons was revealed [15], it became possible to characterize different physiological states by element profiles (metals) [8]. We used both approaches for differentiation between live and dead spores of *A. salina* by physico-chemical methods [8,15]. The possibility of conjugation of nonequlibrium electrochemical processes, determining the rate of interphase transfer of metal ions, with the neutron flow was previously demonstrated [7].

MATERIALS AND METHODS

The device for exposure to neutrons consisted of an INK1-06 neutron source (^{252}Cf, activity $1.42\pm0.11\times10^4$ n/sec (±8%), neutron decelerator (polyethylene), and a cuvette for *A. salina* spores, coaxially encircling ^{3}He neutron counter (type SI14N). The neutron detector was connected to the pulse counter device through discriminator amplifier. The neutron flow density from ^{252}Cf source, equivalent dose power, and energy spectrum were calculated by the Monte Carlo method [13]. Estimations were made with consideration for the real geometry of the device consisting of a 37-cm-high cylindrical polyethylene block 20 cm in diameter with a removable cylindrical cuvette on the axis of the cylinder in a cylindrical hole 5 cm in diameter. The cuvette was made from organic glass and was 18 cm high, 4.8 cm in diameter, with 2-mm-thick walls. It consisted of two coaxial parts: a cylindrical ^{3}He neutron counter (1.8 cm diameter, 15 cm height) on the axis of the central part of the cuvette (external diameter 2.4 cm) at the level of the cuvette mid-height, while the inner part was for *A. salina* spores. The neutrons reached the detector passing through the layer of spore biomass. Radioactive source in a thin-wall steel capsule was placed in a narrow cylindrical opening at a distance of 4.5 cm from the device axis at the level of the middle of removable cuvette. After thermal neutrons passed through the layer of live and dead spores, they were scintillated. The weight of samples was 100.48 g, filling density 0.53 g/cm^3. The

*Laboratory of Applied Hydrochemistry and Analytical Chemistry, State Oceanographic Institute, Russian Hydrometeocenter; **Institute of Pulsed Technology; ***Institute of Physical Technological Problems, Moscow. ***Address for correspondence:*** antonvs@mail33.com. A. V. Syroeshkin

0007-4888/04/1385-0470

neutrons in the entire device (completely assembled) were counted for 300 sec, the being counting repeated at least in 4 repetitions, with the mean relative standard deviation of less than 0.6% at confidence probability of 0.95. Basal neutron count in assembled device (together with spore samples) was no more than 0.05% of the experimental signal.

Analytical weighing of *A. salina* spores was exposed to acid mineralization, and metal content was measured on a SpectrAA-800 atomic absorption spectrometer with electrothermal atomization and Seeman correction of the basal level, as described previously [6]. The relative standard deviation in measurement of the element content at confidence probability of 0.95 did not surpass 20% for Al, Cu, Cd, and 15% for Ni, Cr, Mn, As, and Pb. Three parallel samples were processed and analyzed in all experiments.

The spores were grown in artificial sea water (100-200 spores in 150 ml) of the following composition: 0.46 M NaCl, 28 mM $MgSO_4$, 26 mM $MgCl_2$, 9.2 mM KCl, 6.9 mM $CaCl_2$, 2.3 mM $NaHCO_3$, 0.15 mM $SrCl_2$, 6 μM $MnSO_4$, 24 μM LiCl, 4 μM Na_2MoO_4, 0.5 μM KJ, 3 μM $Al_2(SO_4)_3$, 0.8 mM KBr, 0.7 μM $FeCl_3$, 0.6 μM $CoSO_4$, 1 μM $RbNO_3$, 4 nM $CuSO_4$, 0.4 μM $ZnSO_4$, 0.4 mM Na_2SiO_3, 50 μM NaF, and 23 μM NaH_2PO_4.

The spores (500 g) placed into a glass vessel were thermally killed in a drying box at 150°C for 9 h. No appreciable dehydration of the spores was noted: hydrogen content in live (initial) and dead spores, measured by reaction gaseous chromatography on a CHNS analyzer 1108 (Karl Erba) was 7.3 and 7.2%, respectively (mean standard deviation 0.02).

RESULTS

Energy spectrum of neutron radiation from ^{252}Cf has the maximum in the fast neutron range. Addition of the neutron decelerator (polyethylene layer) to the device led to thermalization of neutrons. The maximum flow density fell on thermal neutrons (Fig. 1, *a*). Summary density of the neutron flow (100%) was about ~20 n/(sec×cm^2), which was about 1000 times higher than the basal neutron flow above the Earth or sea surface [11]. The duration of spore exposure to thermal neutrons was 1 h; the spores were seeded 3 h after irradiation. The equivalent dose power created by the neutron flow in the samples was 1.3 μZv/h (100%). It is a low value, surpassing the level of natural background by no more than one order of magnitude [4, 14]. Therefore, neutron irradiation in our study can be considered as slight from the viewpoint of ionizing radiation exposure of live cells. Exposure of live *A. salina* spores to this neutron flow led to their more rapid development after seeding into sea water: by the 42nd hour of incubation 4 times more naupliuses formed from exposed spores in comparison with intact spores (Fig. 1, *b*). No signs of development were detected in the dead spore sample (0% growth). The neutron count was different in the device with live and dead spores: it was 4% higher in the presence of live *vs.* dead spores (Fig. 2, *a*). The relationship between neutron count and sample weight also differed greatly for live and dead spores (Fig. 2, *b*, *1*, *2*) and did not coincide with the estimated relationship (Fig. 2, *b*, *3*). The latter fact suggests that the interactions between thermal neutrons and live matter are not confined to

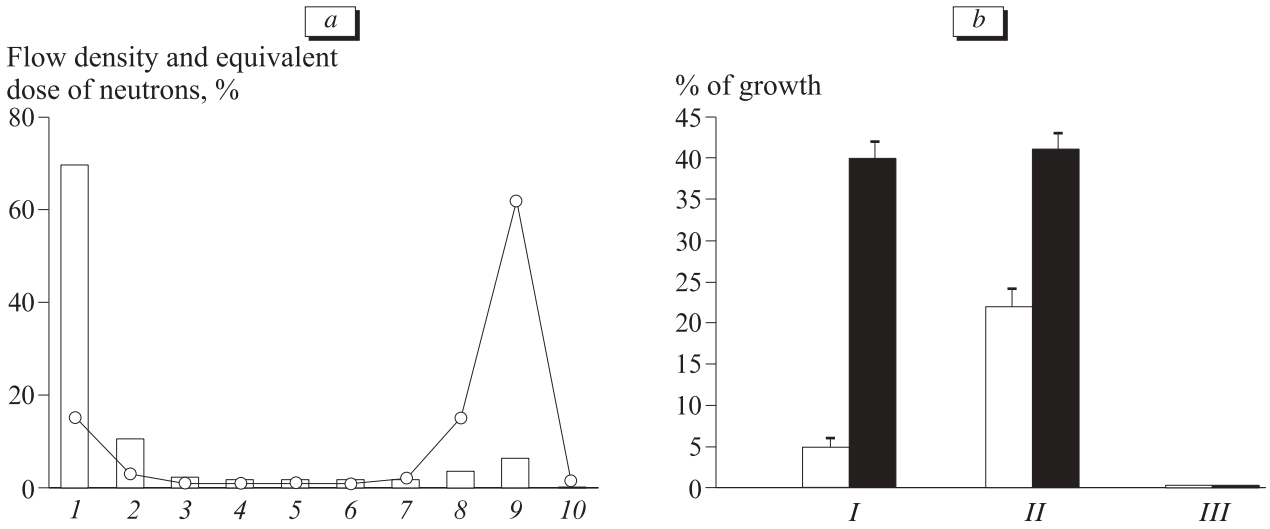

Fig. 1. Effect of exposure to neutrons on the growth of *A. salina* spores. *a*) estimated spectrum of neutron flow density (bars) and equivalent dose power (line with dots) by energy groups (*1-10*). *1*) 0-0.1 eV; *2*) 0.1-1 eV; *3*) 1-10 eV; *4*) 10-100 eV; *5*) 0.1-1 keV; *6*) 1-10 keV; *7*) 10-100 keV; *8*) 0.1-1 MeV; *9*) 1-10 MeV; *10*) 10-100 MeV. *b*) growth percentage. Dark bars: total percentage of sprouting spores and naupliuses *vs.* total number of seeded spores by 42nd hour of incubation in sea water. White bars: percentage of naupliuses in the total number of seeded spores by 42nd h of incubation in sea water. *I*) live spores; *II*) live spore+neutrons; *III*) dead spores.

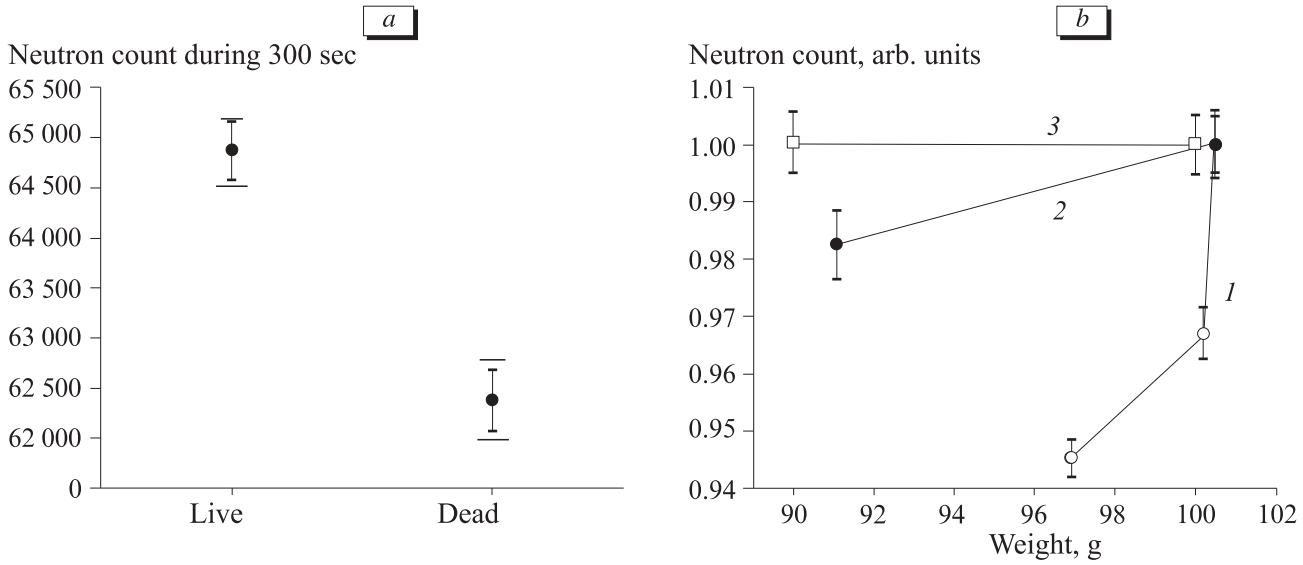

Fig. 2. Interactions between *A. salina* live and dead spores and thermal neutrons. *a*) counts (during 300-sec exposure) after neutron passage through a layer of live and dead spores. Maximum and minimum counts are shown above and below the confidence intervals; *b*) relationship between neutron count and spore sample weight. The following values are taken for a unit of count: *1*) 64 872/300 sec for live spores and *2*) 62 291/300 sec for dead spores. The theoretical relationship (*3*) was calculated from the following averaged element composition of live matter: 10% H, 62% O, 22% C, 3% N, 1% P, and 2% Ca.

their absorption/diffusion on different atoms. Based on the data on conjugation of thermal neutron flow with the unequilibrium electrochemical process [7], we hypothesized that exposure to thermal neutrons modulated the element profiles of live spores, as metal metabolism was largely determined by the values of transmembrane electrochemical potentials. Really, in addition to the biological effect of accelerated development (Fig. 1, *b*), exposure to neutrons for 1 h produced metabolic effects: modulated the element profile ratio for Mn and As (Fig. 3), while the ratios of other elements (Al, Cr, Ni, Cu, Cd, Pb) was unchanged. According to our data, another physiological exposure associated with biological response also leads to specific changes in the element profiles of *A. salina* spores. Cold activation of spore growth (1 month at -20°C) led only to equimolar changes in the Cu and Zn ratio, while the values for other elements remained unchanged.

Interactions of live and dead *A. salina* spores with neutrons were different, which attests to specific conditions of altered status of the substance in live cells (Fig. 2). We think that this was due to transmembrane potentials (Δμ). At Δμ up to 250 mV a gradient of electrical field tension (E) of at least 500 kV/cm forms on the biomembranes of about 5 nm thick. The E values at some sites of the membrane can be higher by 2-3 orders of magnitude, if we take into consideration the surface potentials. This leads to both chemical effects (these E values conform to the conditions for realization of Wien's second effect — superhigh mobility of ions at the expense of "lost" hydrate sheath) and polarization of the nuclei, which, in turn, modifies the type of the substance interactions with neutrons (the neutron nuclear precession phenomenon [1]). The E values of the same orders as on biomembranes manifest by strong intracrystal fields, which leads to alteration of the neutron interactions with the substance [12]. The totality of these neutron optics phenomena helps us to detect a live matter by the characteristics of its interaction with neutron flow (Fig. 2), which is very important for practical problems requiring contact-free detection of live organisms, for example, for bioterrorism control. The relationship between thermal neutron interactions with live matter and the Δμ values opens vistas for neutron tomography, reflecting the metabolic status of organs and tissues. In this study we used thermal neutron flows close to the basal values. Basal flows of thermal neutrons are caused by secondary radiation (result of interactions between solar and Galactic radiation with Earth atmosphere) [3,4] and flows from the Earth crust. Changes in solar activity and gravitation effects of the Moon [3] lead to essential changes in the density of the basal neutron flow. On the other hand, clear-cut correlations between solar activity and functioning of live matter is well known [5,9,10]. The presence of biological (Fig. 1) and physiological (changes in trace element metabolism; Fig. 3) responses to slight neutron flows suggest that neutrons are among the main "messengers" through which live organisms perceive a number of astrogeophysical events.

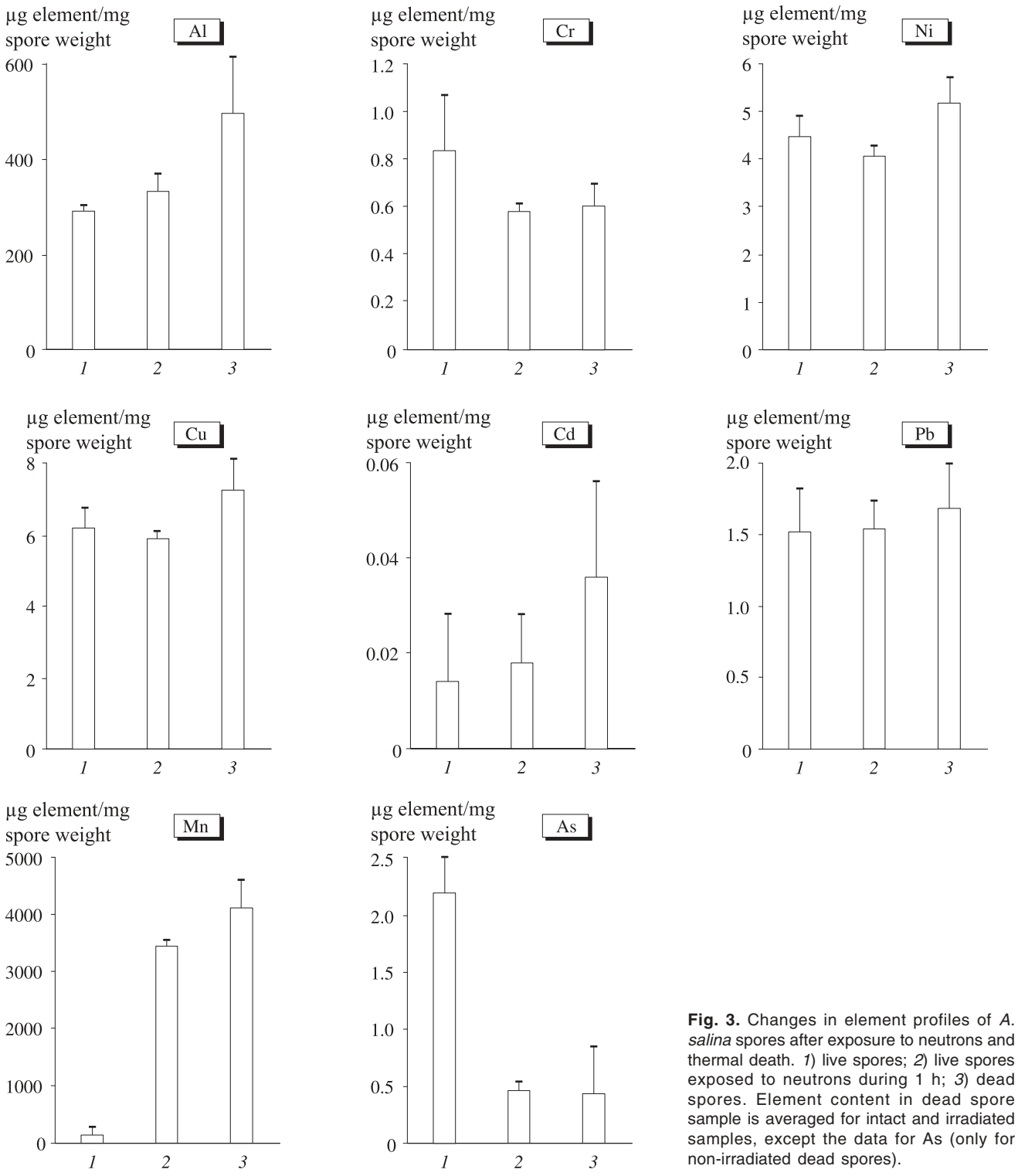

Fig. 3. Changes in element profiles of *A. salina* spores after exposure to neutrons and thermal death. *1*) live spores; *2*) live spores exposed to neutrons during 1 h; *3*) dead spores. Element content in dead spore sample is averaged for intact and irradiated samples, except the data for As (only for non-irradiated dead spores).

REFERENCES

1. V. G. Baryshevskii and M. I. Podgoretskii, *Zh. Eksp. Teoretich. Fiziki*, **47**, 1050 (1964).
2. V. I. Vernadskii, *Chemical Structure of Biosphere of the Earth and Its Environment* [in Russian], Moscow (1987).
3. N. N. Volodichev, B. M. Kuzhevskii, O. Yu. Nechaev, *et al.*, *Astronom. Vestn.*, **34**, No. 2, 188-190 (2000).
4. L. A. Il'in, V. F. Kirillov, and I. P. Korenkov, *Radiation Safety and Protection* [in Russian], Moscow (1996).
5. G. E. Kocharov, V. A. Vasil'ev, V. A. Dergachev, and V. M. Ostryakov, *Pis'ma v Astronom. Zh.*, **4**, 206-210 (1983).
6. V. B. Lapshin, M. Yu. Yablokov, I. S. Matveeva, *et al.*, *Issledovano v Rossii*, No. 118, 1302-1316 (2002).
7. A. G. Lipson, I. I. Bardyshev, V. A. Kuznetsov, and B. F. Lyakhov, *Fizika Tverdogo Tela*, **40**, No. 3, 254-259 (1998).

8. I. S. Matveeva, T. V. Pleteneva, T. L. Berezinskaya, *et al.*, *Mikroelem. v Med.*, No. 3, 15-20 (2003).
9. M. V. Ragul'skaya, O. V. Khabarova, V. N. Obridko, and I. V. Dmitrieva, *Zh. Radioekol.*, No. 10, 1-14 (2000).
10. A. L. Chizhevskii, *Earth Echo of Solar Storms* [in Russian], Moscow (1973).
11. J. M. Carpenter, *Nature*, **338**, 711-716 (1989).
12. V. V. Fedorov and V. V. Voronin, *Nucl. Instr. Meth.*, **B201**, No. 1, 230-232 (2003).
13. J. K. Kim, M. J. Wilson, and J. C. Yanch, *Phys. Med. Biol.*, **38**, No. 8, 1145-1155 (1993).
14. V. Mares, V. Lisa, L. Bacakova, and J. Burian, *Physiol. Res.*, **46**, No. 2, 101-106 (1997).
15. S. Masunaga, K. Ono, I. Sakurai, *et al.*, *Int. J. Radiat. Oncol. Biol. Phys.*, **51**, No. 3, 828-839 (2001).