EFFECTS OF ELF-EMF TREATMENT ON WHEAT SEEDS AT DIFFERENT STAGES OF GERMINATION AND POSSIBLE MECHANISMS OF THEIR ORIGIN

S.I. Aksyonov*, A.A. Bulychev, T.Yu. Grunina, S.N. Goryachev and V.B. Turovetsky

Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow 119899,Vorobyevy Gory, Russia

ABSTRACT

The efficiency of weak ELF (extremely low frequency) EMF on living systems can be explained by taking into account the nonstationary processes that arise when ions pass part of the intermembrane distance during the EMF period. The periodic movement of ions in the heterogeneous medium would result in the nonlinear effects influencing the ionic strength and pH near the membrane and the release of some peripheral proteins to the water phase. Based on this notion, we studied the effects of EMF treatment (30 or 50 Hz, 30 mT) at different stages of imbibition of wheat seeds. The treatment at the stage of activation of esterases increased the leakage of the products of esterase reac- tion with its following retardation, which contrasts to the linear kinetics for untreated seeds and for seeds treated at earlier stages. The treatment also led to a reliable increase in pH near the embryo surface. When the wheat seeds with germinability of 50% were treated at the stage of root formation, a sig-nificant increase in the number of seeds with roots was observed. The sprout length reliably increased after this treatment with respect to seeds treated later and untreated seeds. In the latter case, the number of seeds with sprouts increased only. Long treatment of seeds during the second day of imbibition reduced the length of sprouts. The observed effects are discussed on the basis of this proposed mechanism.

* Corresponding author.

INTRODUCTION

The question on mechanisms of non-thermal effects of extremely low fre-quency electromagnetic fields (ELF-EMF) on various processes in organisms oc-cupies a prominent place in the general problem of the interaction of EMF with living systems. The urgency of this question is determined by the fact that this frequency range includes industrial frequencies, the intensities of which in large cities are several orders of magnitude higher than in the countryside. Furthermore, this range of EMF includes the frequencies of geomagnetic and cosmophysical fluctuations, which have numerous effects on biological systems and even on so-cial phenomena (1-4). This EMF range includes the modulation frequencies that markedly enhance the action of high frequencies on the organism (5). Despite ample data regarding the biological effects of ELF-EMF (1-4), their interpretation and even the obtained results are still the matter of discussion. This is caused, first of all, by the uncertainty about the mechanisms by which the low energy of EMF is transduced within the cell. These mechanisms should provide an explanation not only for the possibility of the biologically significant response to EMF, but also the unusual pattern of relationships that is obtained. The latter is particularly relevant to the mechanism that determined the specificity of the effects of geomag-netic and cosmophysical fluctuations, although high-amplitude treatments do not necessarily lead to similar results. Such relationships are generally characteristic of the EMF effects in organisms, with absence of proportional relation to field intensity and even with the enhancement of biological effect upon the decrease of field intensity (1-4). The dissolving of this paradox would be of principal sig-nificance for the general problem of interaction of EMF with living systems, be-cause the energy of this interaction for extremely low frequencies of EMF is par-ticularly small.

To explain such dependencies of EMF effects in organisms, it is necessary, on the one hand, that the cells possess the mechanisms that ensure the enhance-ment of weak treatments by means of various nonlinear processes as well as con-centrating them on cell structures with a high sensitivity to such effects. On the other hand, the cell should be the site of processes which would be able to restrict or reduce the influence of EMF upon the increase in EMF intensity. These tasks have not been solved in models, that related the effects of EMF with their actions on certain characteristics of membranes (6,7).

Other possibilities arise when one considers the nonstationary processes evoked by alternating EMF within the cell and membrane boundary layer, when the ions driven by electric fields, induced inside the cell, pass only a part of an intermembrane distance during an oscillation period of EMF. Such conditions are, in particular, fulfilled for fields and frequencies of geomagnetic fluctuations. By considering the mobility of K^+ in water at 18^o C [$I = 64.4\ 10^{-4}$ Cm m^2 mol^{-1} (8)], we obtained the result that for the frequencies, typical of such fluctuations, f = 0.1 Hz and lower, and field intensity in the range of 1-10 V/m, K^+ ions would cover a distance of about 1 μm during the period of oscillation; this distance is comparable but somewhat

less than the cell dimensions. According to this view, the values of V/m during an oscillation period for the industrial frequencies should be approximately three orders of magnitude higher, which is on the whole consis-tent with the published data on biological effects of such fields (4). In this case, the periodic movement of ions in a heterogeneous intracellular medium would result in different nonlinear effects and establish ion concentration gradients in the interfacial layer near the membrane. The formation of such gradients is facilitated because of the elevated viscosity of this layer and the existence of additional elect-rostatic interactions within it. In addition, because of the transmembrane selective ion exchange (with an account of the large difference in concentrations of Na^+, K^+, and Ca^{2+} on different sides from the membrane) the pH can also change in this layer. Furthermore, these effects are specific for weak ELF-EMF (9}. In their turn, changes in the interfacial membrane layer would affect the transitions of weakly bound peripheral proteins into water or backward. Because the number of freedom degrees increases upon the transfer to water of certain groups of protein macromolecules, having the dynamic structure in water, these transitions must be associated with little change in free energy (10,11). The relatively long influence during the period of ELF-EMF compared with higher frequencies must promote overcoming of the activation barrier for such transitions. In particular, the dura- tion of influence of ions on electrostatic interactions between peripheral proteins and the membrane apparently determines the specificity of Ca^{2+} effects on cellular processes, because the binding constant with membrane is much higher for Ca^{2+} than for Mg^{2+} (11).

It is known that many peripheral proteins are bound to the membrane by only electrostatic interactions and that the release of such proteins to water can be caused by the elevation of ionic strength of the solution to 0.15 M NaCl (12) or by a change in pH by a fraction of a unit. For example, the increase in pH by 0.5 unit can stimulate cell division (13); furthermore, some stages of cell division continue despite the inhibition of biosynthetic processes (14), which indicates that the structures involved at these stages have been formerly present in the bound state. The transitions of proteins to the cytoplasm under various treatments allow an explanation for numerous data concerning changes in cytoplasm viscosity, light scattering, and distribution of dyes in the cells (11), which occur upon activation of almost any metabolic process (15,16). There is evidence that various weak treatments, including pulse ELF-EMF (17), induce the release of bound proteins. A number of other examples demonstrating the sensitivity of such transitions to various weak treatments were cited in (11,12,18).

At the same time, in the external EMF of higher intensities, ions have enough time to cover all of the distance between the membranes. In this case, the steady- state distribution of the induced voltages is established, according to the resis- tances of the circuit segments. Then, almost the entire voltage drop

induced by the external field would occur across the membrane, where it would constitute only a small fraction of the intrinsic membrane noise. Conversely, for the nonsta-tionary case of weak fields, the voltage induced by the external field is distributed over the cell volume and its relative portion is substantially higher than at the membrane. Moreover, the voltage drop is not of major importance; more significant is the fact that the conductance of intracellular solution is higher than the membrane conductance; this would cause a notable effect on the ion flow even at low fields.

Thus, consideration of the non-stationary processes, related to ion movement in the intermembrane space, provides an approach to elucidation of mechanisms that underlie the unusual dependence of biological effects on the intensity of EMF. The appearance of such effects would depend not on the amplitude but on the ratio between amplitude and frequency of EMF, when extremely low frequencies are combined with the very low amplitude of the field. Transitions of proteins from bound to the free state or backward and changes in intracellular pH may serve as factors that are particularly sensitive to ELF-EMF. The experimental verification of the above considerations was the aim of this study.

It should be taken into account that the results of the experiment substantially depend on the choice of object. The extent of the anticipated effect may depend on the physiological conditions, which is not easily controlled. Within the cell, a large set of various processes takes place, and these processes may oppositely change under EMF treatment. There are other uncontrolled weak treatments, etc., which reduce the reproducibility and reliability of the results.

Based on these considerations and having in mind to study the effects of ELF- EMF, we used wheat seeds at the stage of their imbibition and early stages of germination. Seeds were chosen because their transition from dormancy to germi- nation at early stages is unidirectional -- during this transition, various structures are released and this process is associated with a certain sequence of events: first, roots are formed and, next, the sprouts appear (19). Therefore, by adjusting the time of EMF treatment, it is possible, in principle, to selectively affect different responses. In turn, different sensitivities of various processes to EMF treatment during imbibition and germination of seeds, permit differential measurements, which elevates the reliability of the detection of responses that are specific for EMF. The reliability of detection increases if several parameters are measured and compared. Such parameters include pH changes near the surface of em- bryo and in the bulk solution, the hydrolytic activity of esterases released during imbibition, the kinetics of leakage of esterase activity products, the number of seeds with sprouts and roots, the length of sprouts, and other, less direct biological characteristics. Using seeds of several wheat varieties, which differed in germina- tion ability, these parameters were measured after EMF treatment at different stages of imbibition and germination and compared with similar characteristics observed on untreated seeds.

MATERIALS AND METHODS

Wheat seeds of the cultivar. Zarya with germinability of 95 and 20% and the cultivar Inna with germinability of 80 and 50% were mainly used in this study; seeds of several other wheat varieties with different germinability were also used. The imbibition was performed according to the common procedure on Petri dishes (d = 80 mm) with two layers of filter paper. All seeds in each experiment were soaked in distilled water in the same vessel and before EMF treatment were di-vided on two or more (up to 6) identical samples soaked with 10 ml of water each (treated and untreated seeds). The samples in different types of experiments contained either 10 or 20 seeds each disposed compactly in the random orientation in the center of the disk. At different time after soaking, the test sample was exposed to low-frequency rotating magnetic field (MF) for 7, 10, or 15 min. A MM-5 magnetic stirrer was used as a source of MF. The dish was usually placed on the surface of a stirrer, and in some experiments the dish hung at a different distances above the stirrer. The highest amplitude of MF at the level of a sample was 30 mT ± 20% (peak to peak) at a frequency of 30-33 Hz; the oscillations were close to a sine wave. MF was measured from the induced voltage in the coil as compared with the standard sample.

In other series of experiments, we used a MAG-30-3 device (Instrumental Works, Elatoma, Russia) designed for low-frequency therapy, which is used for medical purposes. The operating frequency of the device was 50 Hz with a slight contribution of the third harmonics. The highest amplitude of MF was similar to that of the magnetic stirrer and measured 30 mT ± 20%. This device was used upon comparing the effects of short-term (15 min) EMF treatment applied 17 and 24 h after soaking and the long-term EMF treatment (during the total second day of imbibition) on processes of root and shoot formation during seed germination. To reduce the thermal effects, the device was cooled by water from outside, and an air gap of about 2 mm was provided between the sample and the device. The highest temperature at the site of the sample location did not exceed 27-28^{o} C, which is in the range of temperatures considered optimal for wheat-seed germina-tion. A similar temperature was maintained in the thermostat, where seeds were placed since the second day of imbibition. To exclude possible differences in the light regime, some experiments were performed in the light at room temperature; these experiments gave similar results. Seeds were treated with EMF in Petri dishes. Each Petri dish contained 20 seeds (this number was limited by the re-stricted area of EMF treatment (diameter of the coil is about 40 mm)). The numbers of seeds with roots and sprouts, as well as the length of sprouts were measured on the sixth day of imbibition.

The activity of esterases during the imbibition of seeds and their changes after treatment of seeds with EMF were determined from the effectiveness of hydrolysis of nonfluorescent compound fluorescein

diacetate (FDA) to fluorescein (Fl) and the leakage of Fl into the medium (20). To do this, 10 seeds of test and control samples were taken at a certain stage of imbibition and washed with water three times for the removal of products that had leaked previously; after that, 3 ml water and 15 µl of 0.5% FDA solution were added to the seeds. After 50 min, 200 µl aliquots were sampled for fluorescence measurements. In addition, the kinetics of leakage of Fl was measured 1 and 2 h after the first measurement. Microfluorometric measurement were conducted on a LUMAM-I3 fluores-cent microscope with a FMEL 1A photometric attachment. Fluorescence was measured from the area 150 µm in diameter. To excite fluorescence, a KGM 9-70 lamp and SZS 21-2 and FS 1-2 glass filters were used. To measure fluorescence, we used a FEU-79 photomultiplier and an interference filter with peak transmit-tance at 520 nm and half-band width of 12 nm.

Changes in pH near the seed embryo during imbibition were measured using a glass-insulated antimony microelectrode with a tip diameter of 10-20 µm; seeds were placed into the same solution where imbibition proceeded. The microelec-trode was positioned using KM-2 micromanipulator; the position was controlled under microscope (21). The tip of the microelectrode was placed at a distance of 5-10 µm from the embryo surface, which allowed detection of local changes that appeared earlier than did changes in the bulk solution. Some experiments were performed on individual seeds during the whole period of imbibition. In other experiments, we measured both the local pH and the «bulk» pH values; the bulk pH was measured at a distance of about 5 mm from the embryo. In these measurements, the effect of EMF treatment was evaluated from the gradient, ΔpH between local and bulk pH values and difference between treated and untreated seeds. With this approach, we could markedly increase the statistics of measure-ments, as compared to experiments on individual seeds during the whole period of their imbibition.

Statistical treatment of results was performed using Statgraphics software and *p* Student's test.

RESULTS

Effect of EMF Treatment on Activation of Esterases

Esterase Activity

We anticipated that the activity of esterases in imbibing wheat would appear at a certain stage of imbibition, when a sufficient level of hydration is reached within the cell and the enzymes are able to transfer from the bound to the free state with an accompanying increase in their activity. It can be also anticipated that at this stage, the sensitivity of this process to EMF treatment would increase. This phenomena indeed took place for the seeds of cv. Zarya with 95% germinability (Fig. 1A). Each point on the curves in Figure

1 represents an individ-ual experiment. Measurements of the leakage of Fl released after hydrolysis of FDA showed that at the beginning imbibition (up to 6--8 h) of control (un- treated) seeds, no significant changes in Fl leakage occurred. At later stages, we observed a sharp increase in the amount of Fl released to the external medium; the peak of the leakage was noticed between 15 and 18 h from the beginning of imbibition. After the peak, the rate of Fl release decreased to about the initial level (Fig. 1A**).**

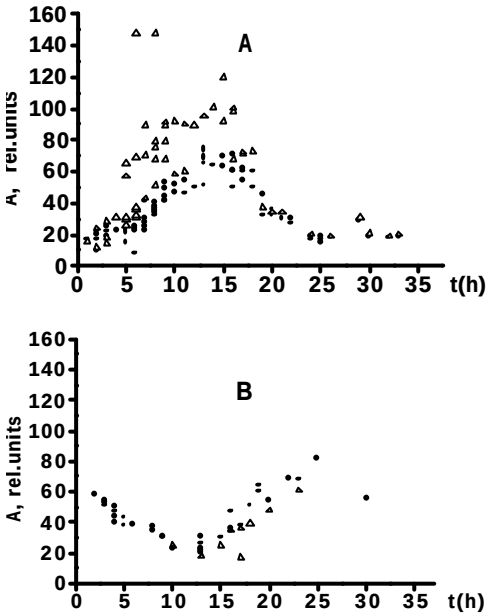


Figure 1. Leakage of fluorescein from wheat seeds of the cultivar. Zarya with high (A) and low (B) germinability at different stages of seed imbibition under control conditions (•) and after 7-min treatment of seeds with ELF EMF (Δ).

Each stage of manifestation of esterase activity corresponds to qualitatively distinct differences in the sensitivity of this activity to 7-min EMF treatment of seeds on a magnetic stirrer MM-5. The largest effects of EMF on seeds with high germinability were noted at the stage of activation of esterases, at a time near the peak of esterase activity; in this case the effect reached the factor of two, although the variability of data in different experiments was quite large. After 11 h of imbibition a pronounced response to EMF treatment was noticed in all experiments, whereas EMF had little effect on the leakage of hydrolysis products after 17—18 h, when the esterase activity decreased (Fig. 1A).

In seeds of the cultivar Zarya with 20% germinability, we observed a different pattern of esterase activity (Fig. 1B). In this case, after a high initial rate of hydrolytic reactions, caused apparently by the release of enzymes and the reaction products from damaged cells, a decrease in the rate of hydrolysis occurred, simi-

larly to seeds of the first group, but 10--12 h later, these seeds exhibited a peak of leakage of hydrolysis products. In these seeds, the EMF treatment either did not change or even decreased the release of FI into the medium (Fig. 1B).

In order to obtain additional information about the origin and features of esterase activation, we performed comparative measurements of the effects of EMF treatment when seeds were either placed directly on the surface of a magnetic stirrer or hanged at a certain distance from the stirrer surface to exclude vibration. In this series of experiments, we determined the dependence of the leakage of Fl on the duration of EMF treatment and the kinetics of Fl leakage at different stages of imbibition.

In seeds of the cultivar Inna with 80% germinability (i.e., intermediate level of germinability compared with two seed lots of cultivar. Zarya), positive effects of EMF treatment on the leakage of FDA hydrolysis products were observed for a wider range of imbibition periods, up to 24 h and even longer. This time range was used for comparative studies. Data from 28 experiments, in which seeds were either placed at the magnetic stirrer or hanged above the surface, showed that the effect of hanging constitutes 0.64 ± 0.03 of the total effect. In other 26 experi-ments, the effect of hanging the sample at distances of 1 and 2 cm from the stirrer constituted 0.68 ± 0.05 and 0.58 ± 0.05 of the total effect of EMF treatment. This decrease can be attributed partly to weakening of the EMF amplitude. How-ever the vibration have the some contribution. In order to additionally assess the influence of vibration, we replaced the rotating magnet with a similarly shaped copper brass disk. After such a replacement, we obtained the effect, which consti-tuted 0.31 ± 0.05 of the total effect. Hence, the main part of the esterase activation (at least about two-thirds of the total effect) was due to the action of EMF, and a smaller part was due to vibration, although in the latter case there was some contribution from the field created by rotating electric motor.

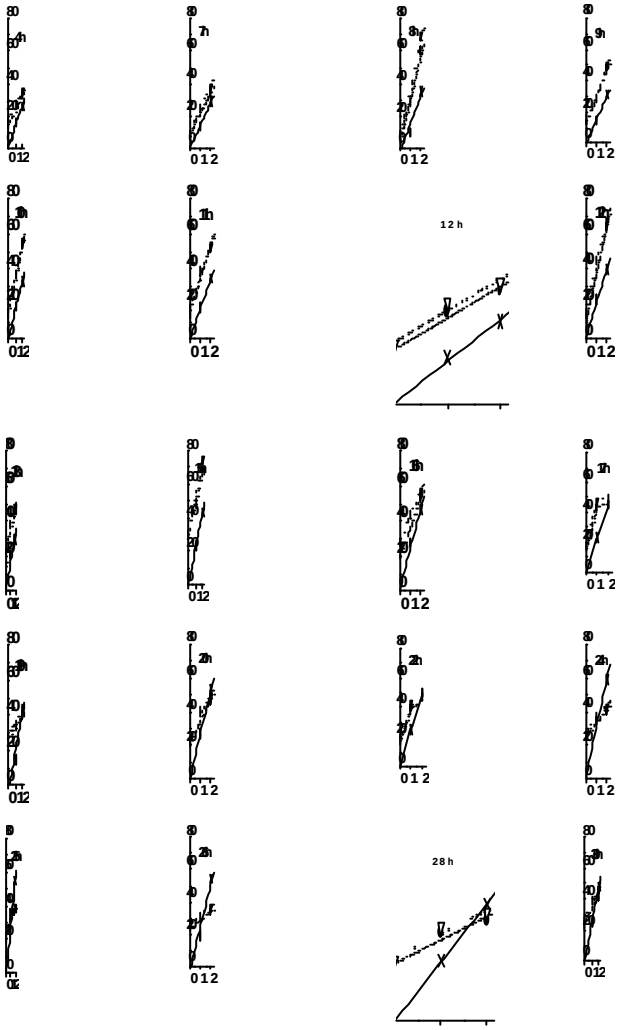
Kinetics of Leakage of Esterase Activity Products

The main role of the effects of alternating EMF was proven in experiments, in which the kinetics of leakage of FDA hydrolysis products were investigated at different stages of imbibition and upon keeping the sample on a thread above the magnetic stirrer or upon placing it directly on the stirrer. We also measured the dependence of Fl leakage on the duration of EMF treatment. The treatment for 7 or 15 min has virtually no influence on the results obtained. The most complete measurements of kinetics were made for 15 min treatment. The data are shown in Figure 2, in which every group of curves corresponds to different hours of imbibition. The kinetic data on the leakage of Fl were compared with the leakage in control experiments, where Fl leakage was measured 50 min after the end of washing of seeds from previously released

products; these data were taken as the origin of coordinates. Other points on curves correspond to the difference between this quantity and the measured values of Fl leakage from untreated seeds and seeds treated with either EMF alone or with EMF plus vibration. In all cases, first measurements were conducted 50 min after washing and next measurements were made 1 and 2 h after the first one.

By treating with EMF, the seeds placed on or above the stirrer, we found that, despite certain quantitative differences, the kinetics of Fl leakage from seeds with germinability of 80% was almost identical for both treatments, but they qualitatively differed from the kinetics of Fl release from untreated seeds (Fig. 2). In untreated samples, at any stage of imbibition (from 4 to 30 h) the kinetics of Fl release was linear. We noted the increase in the total efflux of Fl and the achievement of its peak by the end of the first day of imbibition, i.e., at intermedi-ary times as compared to seeds with germinability of 95 and 20%. At the same time, in both variants of treatment, the linear kinetics was only observed during 12--13 h of imbibition; later on the kinetic pattern strikingly changed. The differ-ence between leakage of Fl from treated and untreated samples increased in the first measurements, but then the Fl leakage from treated seeds decelerated in both variants of treatment, and 2 h later, the leakage became even less than from un-treated seeds. The pattern of such retardation was virtually the same, when EMF treatment was applied alone or in combination with vibration. These data, as well as qualitative and quantitative differences between Fl leak from treated and un-treated seeds, present additional evidence in favor of reliability of the observed effects of EMF, where the key role seems to belong to an electro-motive force induced by the external magnetic field within the cell interior. The retardation of Fl leakage suggests that the measured effect is influenced at later stages of imbibi-tion by the state of membrane structures, which, in turn, depends on EMF treat-ment. This is indirectly evidenced by the lack of microbial growth on the surface of EMF-treated old seeds, whereas such growth normally occurs owing to the leak of organic substances from untreated old seeds. The lack of microbial growth was also observed on wheat seeds with 0% germinability, although active growth of microflora took place in untreated seeds from the same lot. The effect of healing of injuries in membrane structures of seeds was confirmed by a substantial increase in seed germinability and the rate of growth (22).

A (relative units)



time (h)

Figure 2. Kinetics of fluorescein leakage from wheat seeds of cultivar. Inna at different stages of imbibition under control conditions (×) and after 15-min treatment with ELF EMF of seeds posi-tioned either on the surface of the magnetic stirrer (•) or at a certain distance above the surface (∇) as a function of time from the end of seed washing. The leakage of fluorescein was expressed in relative units. Zero corresponds to the fluorescein leakage from untreated seeds, measured 50 min after washing out the seeds. The times of imbibition are shown on the diagrams.

Hence, the treatment of wheat seeds by ELF EMF at the stage corresponding to the activation of esterases results in an obvious acceleration of the leakage of Fl, while weaker effects of EMF were observed at preceding and subsequent stages. The time of the appearance of the effect depends on the state of the seeds and their germinability, as well as on the condition of membrane structures, which is, in turn, affected by EMF treatment. These data are consistent with the suggested mechanism for the action of ELF-EMF -- the release of weakly-bound proteins and their activation in aqueous medium.

Effect of EMF Treatment on pH Changes near the Embryo of Wheat Seeds

Additional information about the mechanisms underlying the effects of ELF- EMF comes from measurements of the pH near the surface of the wheat seed em-bryo. At a certain stage of seed imbibition, apart from the release of bound pro-teins, pH changes occur near the embryo, which are determined by the transfer of protons from the surrounding medium to ensure acidification of the internal medium in seeds (19). In our experiments, we measured the pH upon positioning of a pH-sensitive electrode tip near the embryo surface, outside of the seed; this positioning of the electrode excluded any possible influence of the electrode on the processes investigated. Experiments, performed on individual seeds of the cultivar Zarya with 95% germinability during the total period of imbibition, showed that the external pH decreases during the first few hours, which is presumably due to the leakage of salts from seeds. Later on, a pH increase near the embryo was observed, which occurred first slowly and then (after one-day imbibition) showed a sharp rise (Fig. 3). The beginning of this pH increase varied in control experi- ments for different seeds within the time range of few hours.

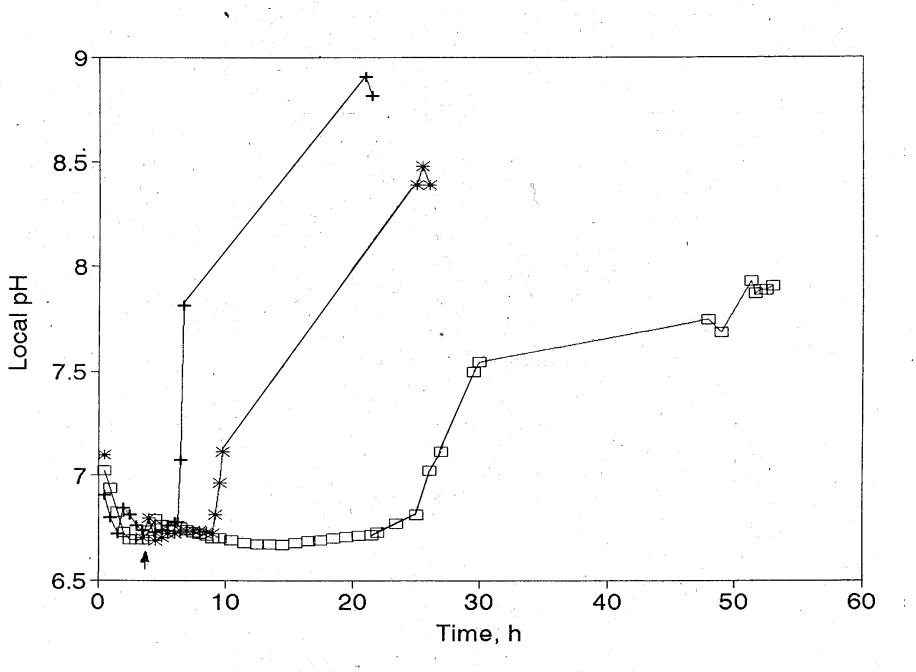


Figure 3. Kinetics of pH changes near the embryo of wheat seeds with 95% germinability (cultivar Zarya) during seed imbibition under a typical control experiment (ð) and 2 experiments with active response on 10-min treatment with EMF of a magnetic stirrer {(✳) and (+)}. The moment of treatment is indicated with arrow.

This process was rather sensitive to 10-min treatment of seeds with EMF during seeds imbibition. However, the response of different seeds was not uniform because of seed heterogeneity; thus, significant acceleration of pH changes at dif-ferent stages of imbibition was only observed in some experiments; this accelera-tion occurred few hours after EMF treatment (Fig. 3). Therefore, to obtain more complete information about influence of EMF treatment on the pH, subsequent experiments were performed not on individual seed during the whole period of imbibition, but on samples consisting of several tens of wheat seeds of cultivar Inna with 80% germinability. These seeds imbibed within the same volume of water and were divided into test and control samples prior to EMF treatment. Table 1 gives measurements of local and bulk pH values on seeds that were treated with EMF for 10-min at various stages of imbibition and continued to imbibe after the treatment (test samples) or were left untreated (control samples). The values represent absolute values of pH in the bulk solution and in local regions near the embryo, the differences between these values (ΔpH for test and control samples). The last three columns show differences between bulk pH for treated and untreated seeds (ΔpH bulk), between local pH for treated and untreated seeds (ΔpH local), and the difference between these values, Δ(ΔpH). Linear changes of pH with time between control measurements were taken into account. The stan- dard error of the mean was calculated separately for two absolute pH values and for the differences between bulk values and local pH values.

Table 1 The Change of pH Near Surface of the Embryo during the Imbibition of Wheat Seeds

Time of EMF Treatment (h)	Time of Measur.(h)	Number of Seeds	ðÍ Bulk Control Experiment		ðÍ Local Control Experiment		ΔðÍ (Local - Bulk) Control Experiment		Effect (experiment - control) ΔðÍ Bulk	ΔðÍ Local	Δ (ΔðÍ)
5 h	8 h	10 (control)	7.40±0.08		7.15±0.04		-0.25±0.06				
	9 h	10 (exp)		7.54±0.08		7.18±0.06		-0.35±0.14	-0.14±0.12	0.03±0.07	-0.10±0.15
9h	13 h	10 (c)	6.93±0.03		7.04±.03		0.11±0.02				
	14 h	10 (exp)		7.02±0.03		7.16±0.06		0.14±0.03	0.09±0.05	0.12±0.07	0.03±0.04
12 h	16 h	10 (c)	6.88±0.05		7.08±0.08		0.20±0.07				
		5 max	7.00±0.02		7.28±0.05		0.36±0.10				
	17 h	9 (exp)		6.94±0.02		7.35±0.06		0.41±0.05	-0.08±0.04	0.17±0.08	0.16±0.09
		5 max		6.99±0.02		7.48±0.05		0.50±0.04	-0.05±0.04	0.12±0.08	0.12±0.11
	18 h	9 (exp)		7.03±0.03		7.51±0.07		0.48±0.06	-0.01±0.04	0.22±0.09	0.18±0.08
		5 max		7.09±0.04		7.63±0.10		0.59±0.06	-0.02±0.05	0.18±0.12	0.19±0.08
	19 h	10 (c)	7.06±0.03		7.39±0.06		0.34±0.05				
		5 max	7.16±0.02		7.53±0.05		0.42±0.04				
17 h	19 h	5 (c)					0.29±0.12				
	20 h	10 (exp)						0.23±0.03			-0.07±0.15
	21 h	5 (c)					0.32±0.09				
6 h	22 h	10 (c)					0.62±0.04				
		8 max					0.63±0.05				
		6 max					0.68±0.06				
	23 h	10 (exp)						0.67±0.12			0.05±0.13
		8 max						0.78±0.11			0.15±0.12
		6 max						0.92±0.06			0.24±0.08
17 h	22 h	5 (c)	7.00±0.02		7.41±0.05		0.40±0.05				

		3 max	7.02±0.02		7.47±0.06		0.45±0.06				
	23 h	9(exp)		7.22±0.02		7.95±0.12		0.68±0.11	0.05±0.03	0.30±0.13	0.20±0.12
		6 max		7.25±0.02		8.12±0.07		0.87±0.08	0.07±0.03	0.42±0.10	0.35±0.11
	24 h	5 (c)	7.34		7.88±0.04		0.55±0.05				
		3 max	7.34		7.93±0.04		0.59±0.07				
		10 (c)	7.17±0.02		7.65±0.05		0.48±0.07				
		6 max	7.18±0.02		7.70±0.06		0.52±0.07				
20 h	22,5 h	5 (c)	7.14±0.08		7.56±0.09		0.41±0.06				
		3 max	7.22±0.12		7.66±0.12		0.53±0.09				
	24 h	8 (exp)		7.71±0.06		8.33±0.09		0.62±0.09	0.41±0.08	0.49±0.15	0.08±0.12
		6 max		7.77±0.05		8.47±0.09		0.70±0.11	0.41±0.10	0.44±0.12	-0.04±0.16
	25 h	5 (c)	7.45±0.05		8.13±0.20		0.67±0.21				
		3 max	7.51±0.07		8.37±0.09		0.95±0.04				
		10 (c)	7.30±0.05		7.84±0.12		0.54±0.11				
		6 max	7.36±0.08		8.03±0.08		0.74±0.11				

It is apparent from Table 1 that, during the first hours of imbibition, acidi- fication of the medium occurred due to the leakage of salts and other substances from seeds. As a result, after 8 and 9 h of imbibition, the local pH was lower than the bulk pH values, both in control and treated samples. No effect of EMF treatment was observed after 9-h imbibition. Later, a slight increase in pH was observed. The effect of EMF treatment also appeared after 12 h of imbibition. At this stage local pH near the embryo was notably higher than the bulk values, in both control and treated samples. The response of individual seeds on EMF treat- ment was not uniform; this is evident from the selection of five seeds from the sample with the highest local pH in the control and treated samples (see Table 1). EMF treatment had virtually no effect on the bulk pH, which is in contrast with its effect on local pH. Five and six hours after the EMF treatment, the change in pH was already higher than the standard error of measurements, both for the total sample and for the group with the best pronounced response, although the effect observed was not yet statistically reliable. When seeds were treated with EMF after 17 h of imbibition and pH measure-ments were performed 3 h after the treatment, no appreciable effect of EMF treat-ment was observed. EMF treatment had a weak influence, when it was applied 6 h after the beginning of imbibition; this weak effect was apparent even 23 h after the beginning of imbibition. However, in this case, the groups of eight and six seeds were distinguished, which showed a stronger response to EMF treatment as compared to the control groups with the same number of untreated seeds dis-playing the maximal difference between local and bulk pH values (Table 1).

In the next series of experiments, EMF treatment was applied after 17 h of imbibition, but the period of observation was longer than in the aforementioned experiments. In this case, 23 h after the beginning of imbibition, pH changes were observed not only near the seed embryo but also in the bulk solution, though the pH changes in the bulk phase were still rather small. At the same time, local pH changes after EMF treatment in six seeds with the highest pH values were 0.4 units higher than in a group of six untreated seeds; this value is obviously higher than the threefold error of measurements ($p<0,02$).

Finally, measurements at 24 h after beginning of imbibition, after EMF treat-ment at 20 h, and upon positioning seeds at a certain distance from the magnetic stirrer, revealed even more obvious changes. At this stage, the response of seeds became more uniform and pH changes occurred both near the embryo and in the bulk solution. In both cases, these changes exceeded 0.4 pH units and were clearly higher than the threefold error of measurements ($p<0,02$ and $p<0,01$). It should be noted that the absolute pH values after EMF treatment were also significantly higher than the control values, although the measurement on a control series was fulfilled 1 h later.

Hence, the short-term treatment of seeds with ELF-EMF results in an obvi- ous acceleration of pH

changes during germination of seeds, this acceleration occurs with a delay of a few hours after EMF treatment. This delay might be caused by the utilization of cell-wall resources (buffer capacity) or other reasons. The responses of individual seeds at early stages of imbibition are not uniform, but these variations were smoothed by the end of the first day of imbibition, and almost all seeds became sensitive to EMF treatment, including the treatment of seeds positioned above the magnetic stirrer, without touching it.

Specificity of Effects of EMF Treatment at Different Stages of Imbibition and Germination of Wheat Seeds.

The various nature of processes at different stages of imbibition and germina- tion of seeds should determine the diverse efficiency of EMF treatment at these stages, including the possibility of both stimulation and suppression of metabolic processes after such treatment. To study the possibility that EMF may induce oppositely directed effects we used the seeds of wheat cultivar Inna with a 50% germinability. In 50 series of experiments, seeds were treated with ELF-EMF (50 Hz, 30 mT) for 15 min after 17 h imbibition; in 34 series, seeds were exposed to a similar treatment after 24 h of imbibition, and in 20 series of experiments seeds were exposed to prolonged EMF treatment during the total second day of imbibi- tion. The data obtained were compared to the results of 59 series of exper-iments with untreated (control) seeds. Some control experiments were common to different variants of EMF treatment, performed during the same days.

Figure 4 shows the distribution of seeds having roots or sprouts under control conditions and after EMF treatments. It can be seen that the number of such seeds in different experiments varied within a wide range. With an average number of about 10 sprouts out of 20 seeds placed in a Petri dish, the number of sprouting seeds in different variants varied from 4 to 17, and the number of seeds with roots varied from 4 to 18. All seeds with sprouts had also roots, whereas other seeds had only roots without sprouts. The mean values obtained and the statistics of data variability in control experiments and in different EMF treatments are presented in Table 2.

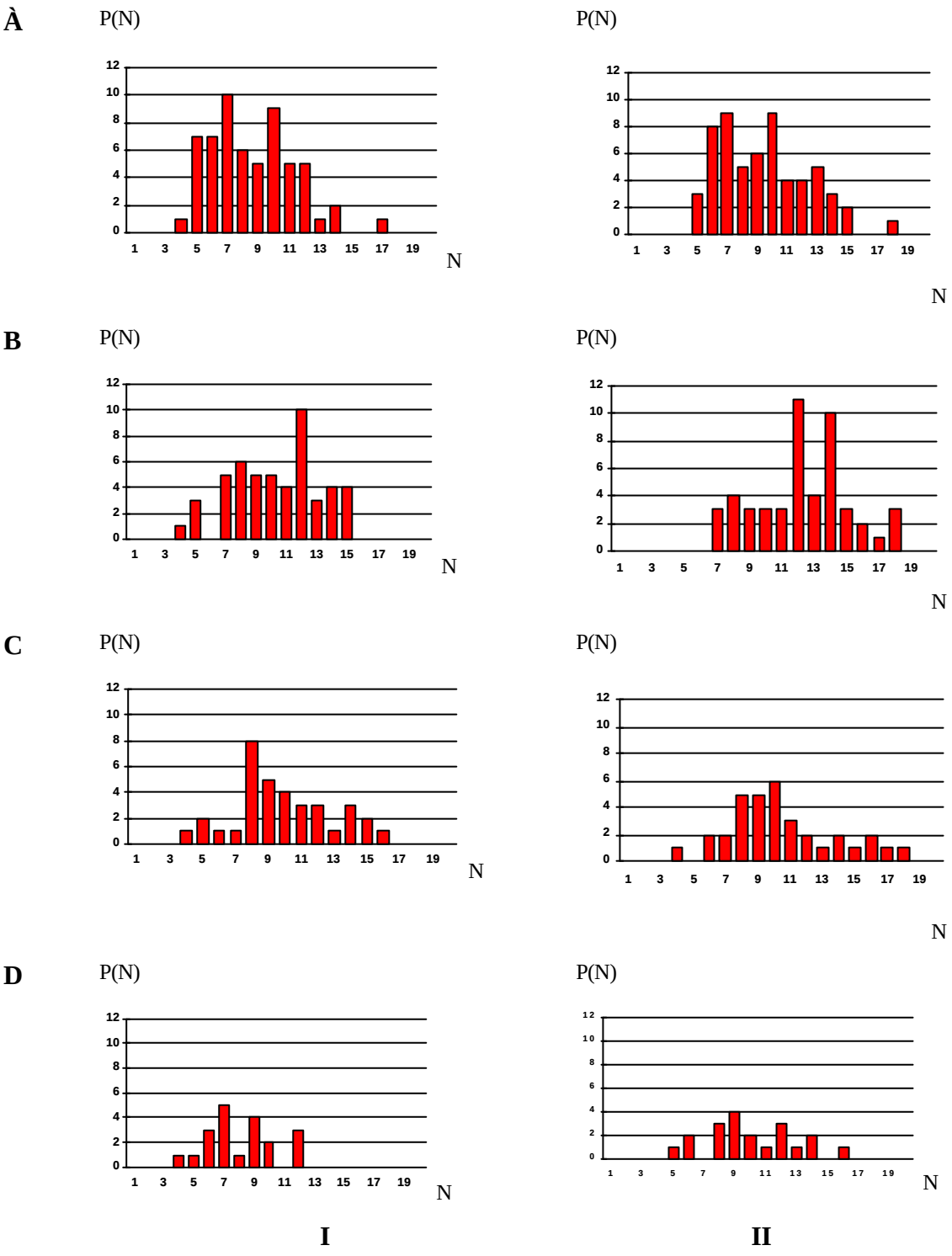


Figure 4. The distribution P(N) of wheat seeds (cultivar. Inna, 50% germinability) by the number N (of total 20) representing the seeds that gave rise to sprouts (I) or roots (II) on the sixth day of imbibition in untreated seed samples (A), in samples treated for 15 min with ELF-EMF after 17 h (B) and 24 h (C) of imbibition,and in seed samples exposed to prolonged EMF treatment throughout the second day of imbibition (D).

Table 2 The Mean Numbers of Wheat Seeds (Out of 20 Seeds) Having Sprouts and Roots in Control Samples and in Seeds after EMF Treatment during Different Stages of Imbibition

	No of seeds with sprouts	Difference from control	No of seeds with roots	Difference to control
Control samples	8,59 ± 0,35	–	9,59 ± 0,40	–
17 h of imbibition	10,32 ± 0.40	1,73 ± 0,55 ($p < 0,01$)	12,30 ± 0,37	2,71 ± 0,55 ($p< 0,01$)
24 h of imbibition	10.00 ± 0,52	1,41 ± 0,63 ($p < 0,05$)	10,44 ± 0,56	0,85 ± 0,68
During second day of imbibition	8,10 ± 0,73	- 0,43 ± 0,81	10,05 ± 0,68	0,46 ± 0,79

It is apparent from Table 2 that EMF treatment after 17 h of imbibition, i.e. when root formation begins, results in a statistically reliable increase in the number of seeds with sprouts ($p<0,01$) and even higher increment in seeds with roots ($p<0,01$) compared with untreated seeds. EMF treatment after 24 h imbibition had weaker effects, although EMF effect on sprouting was still comparable to that observed in seeds treated after17 h imbibition. At the same time, the effect on roots was nearly threefold weaker than the effect observed after 17 h imbibition; it was hardly beyond the range of data scattering. Nevertheless, a prolonged exposure of seeds to EMF at a later stage--during the second day of imbibition--has virtually no effect on the number of seeds with roots but caused an appreciable, but statistically insignificant decrease in the number of seeds with sprouts (sprouts appear later than roots). The latter effect was more pronounced (-1.30 ± 1.00), when control data were compared with the treatment data obtained on the same days rather than to data of all measurements.

Similar, even more pronounced trends were observed for another characteris-tic, i.e., the length of sprouts on the 6th day of germination (Fig. 5). Because of the scatter in sprout length in different series of experiments, all results are presented in relative units with respect to mean values selected as reference points for untreated seeds in each series of measurements. Data presented in Figure 5 for 17 h and 24 h EMF treatment correspond to the results of measurements per-formed on the same days. It can be seen that the greatest influence on the length of sprouts is exerted by EMF treatment performed after 17-h imbibition. The ac-celerated formation of roots influenced the length of sprouts; the average length of sprouts at this stage constituted 26.86 ± 0.41 relative units compared with 19.42 ± 0.41 for untreated samples and 22.46 ± 0.45 relative units for seeds treated after 24-h imbibition. The difference between

these values far exceeds the threefold error of measurements
($p < 0.01$).

Hence, there is a clear stimulating effect of EMF treatment when EMF was applied after 17 h of imbibition; the extent of stimulation far exceeds the threefold range of data scattering for sprout lengths ($p<0,01$). The effect of EMF applied after 24-h imbibition is markedly weaker; moreover, in this case we noticed a broader distribution of sprout lengths, which was caused by heterogeneity of seeds, differing in the beginning time of their germination.

At the same time, the EMF treatment during the second day of imbibition, when the roots and sprouts started their protrusion and elongation, caused a marked suppression of growth. Comparison with control data obtained in the same series of measurements shows that the average length of treated sprouts was 15.04 ± 0.62 rel. units, whereas the length of control sprouts was 19.42 ± 0.59 rel. units. The difference between these values is significant at $p < 0.01$.

Thus, the treatment of wheat seeds with ELF-EMF results in strikingly dif-ferent effects at various stages of imbibition and realization of the genetic program of germinating seeds. It is obvious that the onset and realization of this program is related to the sequential release of various proteins from the bound state and the interaction of the released proteins with various genes during imbibition. At these periods, the proteins become sensitive to EMF treatment. The observed stim-ulation of growth is the result of such interactions caused by EMF treatment. EMF treatment at the early stage of root formation elevates the number of seeds with roots by about one-fourth, which, in turn, stimulates growth of sprouts, so that treated sprouts become 40% longer as compared to control sprouts. These effects are less pronounced at a later stage of growth and development, when the develop-ment of sprouts begins. In this case, EMF treatment affected the number of seeds with sprouts and caused an increase in the average length of sprouts. In addition, the scatter of data was high, which was presumably caused by the heterogeneity of seeds, differing in their germinating capacity.

Finally, the suppression of sprout growth after prolonged treatment with EMF at a later stage of germination should be noted. At this stage, the active growth of roots and sprouts occurs; this growth proceeds via cell elongation and cell division; moreover, cell division is associated with assembling and disassem-bling of supramolecular structures (14). The observed effect of growth suppression upon the prolonged EMF treatment of seeds at this growth stage lent support to the notion that the effects of EMF on the release and binding of various structures differ in their direction. On the whole, this would result in the suppressive rather than stimulating effect of EMF on germination of wheat seeds.

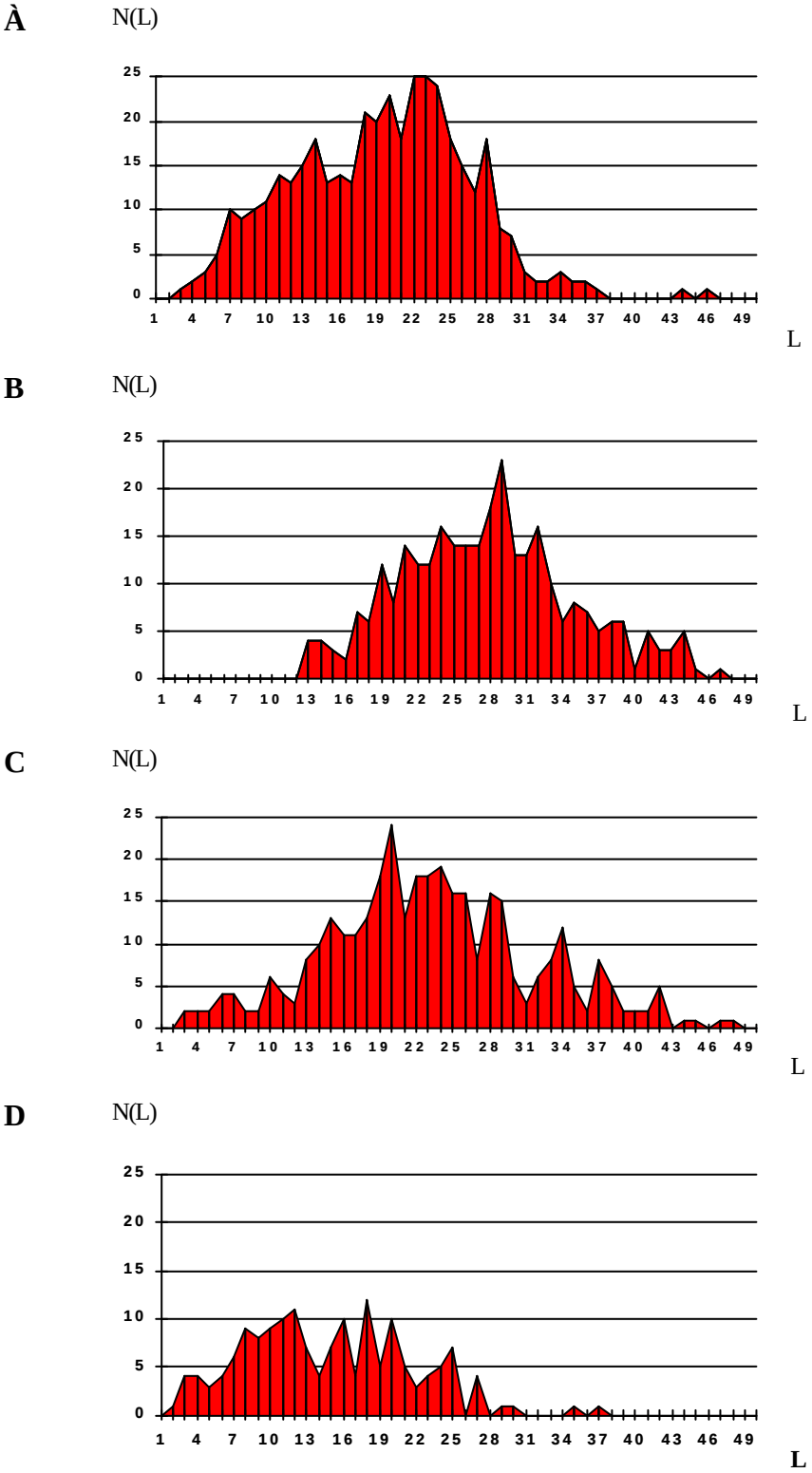


Figure 5. The distribution P(L) of wheat sprouts by their length L (expressed in relative units) on the sixth day of imbibition of wheat seeds (cultivar Inna, 50% germinability) in untreated seed samples (A), in samples treated for 15 min with ELF-EMF after 17 h (B) and 24 h (C) of imbibition, and in seed samples exposed to prolonged EMF treatment during the entire second day of imbibition (D).

DISCUSSION

We must first consider the reliability of the observed effects of ELF-EMF, whereupon the EMF itself plays a more significant role than vibration. The reliabil-ity of the effects of EMF is indicated not only by substantial differences between the records for treated and untreated seeds (these differences clearly exceed the errors of measurements), but also by the various pattern of EMF effects at different stages of imbibition and germination of wheat seeds, which is consistent with different types of biological processes. This includes (a) different sensitivity of seeds to EMF during seed imbibition, (b) the existence of both stimulation and inhibition of biological processes according to their properties at different stages of germination, (c) qualitatively different kinetics of the leakage of reaction prod-ucts from untreated and treated seeds, etc. Finally, all ELF-EMF effects observed are, at least, not contradictory to each other, are consistent with the suggested mechanism of EMF action on living systems, and do not require any additional assumptions.

Our data show that EMF treatment enhances the esterase activity at that particular stage when cells attain a sufficiently high degree of hydration, and the equilibrium in free energy between bound and free forms of certain proteins is nearly established. As a result of such changes, esterases are released at this stage, and even a slight EMF treatment, enhanced by nonlinear effects, may stimulate the release of the enzyme. The relation between the activation of esterases and their release from the bound state agrees with our data about high rate of FDA hydrolysis at the initial stage of imbibition of old seeds (Fig. 1B), when the influx of water is accompanied by the leakage of esterases from damaged cells to the intercellular space. It seems likely, that the release of esterases accounts also for the appearance of the new peak of esterase activity in old seeds. This peak appears with a delay of 10-12 h as compared with seeds with high germinability, because the formation of equilibrium conditions in cells of old seeds would occur with a certain delay, after the completion of the recovery processes. It should be noted that in seeds with intermediate levels of germinability (Fig. 2), the peak of Fl leakage was attributed to the intermediate range of imbibition times compared with the aforementioned seed kinds.

Probably, there is an indirect relation between the release of proteins and changes in the permeability of membrane structures after EMF treatment at this or a later stage of imbibition. This would explain the time-dependent retardation of Fl leakage from the cells after EMF treatment. Based on the pH measurements data near the seed embryo, the opposite effect -- an increase in Fl leakage -- could be anticipated, since the internal seed medium is additionally acidified (20) with a certain lag time after EMF treatment (Table 1). The EMF-induced reduction in membrane permeability and restoration of the barrier properties of

damaged membranes in old seeds would restrict the leakage of other substances that can be normally utilized by microflora. This would also explain the EMF-induced increase in germinability of old seeds (22), the effect that is largely dependent on the state of membrane structures (23-25). The mechanism of EMF effect on the barrier properties of the membrane, mediated by the release of membrane proteins and, possibly, supramolecular structures, is actually analogous to the ef-fect of increase in cytoplasmic viscosity. Such an increase in viscosity is observed, for example, after injury to cell membrane caused by the insertion of microelec-trode; the increase in viscosity of the cytoplasm is probably involved in healing of the membranes after such an injury (15,16). It should be noted that EMF exerts its effect on barrier properties of the membrane at that particular stage when quasi-equilibrium conditions are established within the cell and when the increase in the Fl leakage and the subsequent retardation of the Fl leak are evident after EMF treatment (Fig. 2).

Morever, the effect of stimulated protein release could explain the influence of EMF treatment on the number of seeds with roots and sprouts, as well as on the length of sprouts, when EMF treatment was applied at certain stages of seed imbibition. Although the beginning of these processes is not precisely determined (only later stages associated with the appearance of roots and sprouts can be detected), it is obvious that each of the processes starts earlier and involves the protein that were formerly present in seeds in their bound form. During imbibition, the proteins are released and become sensitive to EMF treatment; further-more, different times of imbibition correspond to different effects of stimulation of root or sprout growth. In addition to these proteins, other structures are released and facilitate the restoration of membrane barrier properties; consequently, after EMF treatment at a stage of root formation, the number of seeds with roots in-crease by about one-fourth. On the other hand, root growth promotes the growth of sprouts and the increase of sprout length by 40% with respect to control sprouts. Effects of EMF treatment were weaker, if this treatment was applied at a later stage, when the sprout formation commenced. In this case, EMF treatment affected only the number of seeds with sprouts and caused a weaker increase of their aver-age length; moreover, in this case, the scattering of data was rather wide, which was probably determined by the heterogeneity of seeds differing in their germina-bility and the onset time of germination. The latter provides an explanation for a certain, though statistically insignificant, increment in the number of seeds with roots after EMF treatment at this stage.

The proposed mechanism of EMF action underlies the phenomenon of the suppression of sprout growth during prolonged treatment of EMF at a later stage, i.e., during the second day of seed imbibition. Under these conditions, EMF has virtually no effect on the number of seeds with sprouts and roots, since the initial processes have been already triggered, and the active growth of sprouts and roots is in progress. It is

known that processes of cell division during growth include several stages, at which both assembling and disassembling of various supramo- lecular structures (e.g., microtubules) takes place (14). The observed suppression of sprout growth under prolonged EMF treatment of seeds at this stage of germina-tion suggests that effects of EMF on assembling and disassembling of various supramolecular structures differ in their direction. On the whole, this may disturb the synchronism of cell divisions, which would lead to suppression, rather than stimulation, of wheat seed germination under the action of EMF.

This mechanism of EMF effect on biological processes is consistent with one more phenomenon - a clear acceleration by EMF of local pH changes near the embryo, and in the bulk phase at later stages. This process becomes evident several hours after EMF treatment, and at later stages of imbibition as compared with the activation of esterases. This delay can be related to the peculiar features in the evolution of pH changes, where the total response of numerous cells having different initial conditions is manifested. This delay might be also influenced by possible utilization of cell-wall resources (the cell wall medium is acidic), and some influence may come from the scutellum (19). All these factors determine a large variability of data for different seeds, especially, at comparatively early stages of imbibition. Nevertheless, even in this case, the pH changes can only proceed after the cells accomplish formation of equilibrium conditions and become sensi-tive to weak EMF treatments.

Finally, it should be noted that our data about the influence of EMF treatment on wheat seeds are important not only for understanding the mechanisms by which EMF affects living systems. These data can also be useful for studying the molecular and physico-chemical mechanisms of the triggering of germination at early stages when there is no apparent indications for the occurrence of such processes.

Our data showing that biological effects of EMF depend on the creation of quasi-equilibrium conditions inside the cells are consistent with the results of other research-ers. These data suggest that the effects of ELF EMF are sensitive to the metabolic status of cells (26, 27). Apparently, different treatments shift this balance to one or the other direction, with the resulting increase or reduction in sensitivity of the processes under study to EMF.

Thus, our data are in a good agreement with the proposed mechanisms of ELF- EMF action on biological processes. The EMF, a rather weak treatment exerts its effect throughout the cell volume, but eventually its influence is concentrated in narrow boundary layers near the membranes and is additionally enhanced be-cause of various nonlinear phenomena. This results in alteration of the ionic strength and pH with the subsequent release or binding of proteins immobilized on the membranes, which affects the metabolic activity. This physico-chemical mechanism provides the basis for interpretation of paradoxical dependencies of EMF-induced effects in organisms, such as the sensitivity to geophysical and cos-

mophysical fluctuations. This mechanism provides also the basis for interpreting the biological effects of EMF of industrial frequencies. These effects are observed under field strengths that are three order of magnitude higher than geomagnetic fluctuations (4), in accordance with the frequency ratio of these EMF. Hence, the nonthermal biological effects of ELF-EMF are not directly related to EMF energy, which may differ by several orders of magnitude at different frequencies. The suggested mechanism offers an approach to interpretation of both stimulating and inhibitory effects of ELF-EMF. The inhibitory effects may result from desynchro-nization of complex multistep processes, particularly in the case of prolonged treatment with EMF, which may cause opposite alterations at different stages of such a process.

This mechanism does not exclude the existence of other EMF-induced ef--fects, particularly, in the high-frequency range.

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