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PLANT SEEDS IN BIOLOGICAL RESEARCH IN SPACE

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In biological studies, a very important methodical prerequisite is the correct selection of the test subject, which determines the effectiveness of the planned experiment to a great extent. In this case, any biological model should have a broad information content and high sensitivity to the environmental factors studied. However, procedural difficulties sometimes force adherence to this principle to be abandoned. This concerns even the study of the effect of space flight factors on a plant organism. /82*

Airdried plant seeds have most often been used for experiments in space. It is known that, in connection with the reduced amount of matter, the response of the seeds to environmental factors is slowed down. Thus, the radiation sensitivity of seeds is 2-3 orders of magnitude less than that of seedlings or growing plants. However, exposures of air dried seeds in space flight conditions does not involve additional equipment or packaging, and it does not require the creation of special conditions for the vital activities of the seeds, which are well preserved for a long time. Moreover, there is a large number of individuals in the small volume of the packing, the physiological state of the seeds can easily be regulated during a flight, etc. All the abovementioned is of great importance for a space experiment. Therefore, airdried seeds have been exceptionally widely used in studies under space conditions. Seeds of plants of more than 20 different species have been in space. Of the grain crops, wheat and barley, onion and carrot of the vegetables, Arabidobsis thaliana of the wild plants, etc. have been particularly widely represented. Of the radiation sensitive species of seeds, peas and pine have been

*Numbers in the margin indicate pagination in the foreign text.

used in experiments. Their critical radiation dose is a few kilcrads (krad). Experiments with radiation resistant plants, radish and white mustard, the seeds of which withstand a gamma ray dose of up to 200 krad, have been conducted in space.

There is interest in the question of what structure in airdried seeds of slightly active systems is affected by environmental factors and how it transmits information on plant growth processes. There is no doubt that, of the total number of cells (several hundred million), no more than 100 meristem cells in the primary plant growth points acquire information on outside conditions [9]. The meristem cells in dormant seeds usually are in the g_1 phase of cell division. After seeds are wetted, the primary vital activity processes of the meristem cells occur simultaneously. The information accumulated in these cells is rapidly released and can be determined by various indicators. Thus, the effect of space flight factors on a seed is determined by morphophysiological, cytogenetic, biochemical and other characteristics, which are widely used in analysis of the effect of flight on /83 the plant organism. In judging from the literature data, changes in the growth processes of seedlings grown from test seeds [7, 8], as well as chromosome rearrangements in the primary rootlets, [13] have been studied most often. Biochemical criteria have proved to be less successful. They have shown a significant deviation between variants in rare cases [36, 44].

The total number of experiments conducted with plant seeds in space is quite substantial. However, the seed exposure conditions, as well as the methods of study of material, have been different. This has greatly hindered comparison of the data obtained. A number of authors have attempted to correlate the published results on morphophysiological [6] and cytogenetic criteria [27], and to show how extensively studies are being conducted on seeds under space conditions.

In the survey conducted, we have attempted to correlate the results of our own tests, obtained together with colleagues of the Institute of Biomedical Problems, USSR Ministry of Public Health, as well as the data of a number of other studies published in the scientific literature. The experimental material was not analyzed by individual characteristics, but by the effect of individual space flight factors on the seed. The effect of three basic factors cosmic radiation, vibration and weightlessness), which occur in any space flight, have been discussed in greater detail in this survey. Living organisms on the earth are reliably protected from cosmic radiation by the layer of the atmosphere. It is practically impossible to completely protect test subjects from cosmic rays beyond the atmosphere. During exposure in space, seeds are unavoidably exposed to an increased radiation background, and it can be assumed that cosmic radiation has a certain effect on them. However, the strength and direction of the response of the seeds prove to be extremely contradictory. Thus, increased germination of seeds after a flight has been established in grain crops [5], lettuce [41], onions [12], etc. The vital activity of old, poorly germinating seeds is restored after a flight as a rule [5].

A number of authors note that seedlings of test seed which are in space grow significantly better. For example, the green mass of seedlings germinated from lettuce seeds exposed aboard the Kosmos-110 AES [artificial earth satellite] was 50% higher than in the ground control, and the accumulation of dry mass increased by 15% [7]. In our experiments with lettuce seeds aboard the Kosmos-368, Kosmos-782 and Zond-8 AES, a 5-10% increase in accumulation of biomass by lettuce seedlings was established [21]. A change in the biochemical composition of the seedlings was noted. Thus, it was determined in an experiment conducted aboard the Kosmos-110 AES that the vitamin C content of lettuce leaves increased by 20% [7]. Thus, much data has been accumulated which indicates a positive effect of the space flight factors on dry seeds. However, th s is observed in far from all experiments. The variable nature of the space effect has been noted by many investigators [9, 28]. A depressing effect of the space flight factors on experimental seeds also has been found [4]. It is characteristic that the criteria which determine the structural changes in test seeds are still more convincing evidence of the unfavorable effect of space flight factors on their structure. Thus, in 42 space experiments conducted in the Soviet Union with dry seeds, an increase in chromosome rearrangements in the meristem cells of the primary root was noted in 90% of the cases [28]. Contrary data of cytogenetics and physiological criteria introduced some confusion into interpretation of the space effect. It can be suggested that we are dealing here with different manifestations of one process. As was shown in our preceding studies [16, 17], small doses of ionizing radiation activate the self-protection response of the plants, which contributes to an increase in recovery processes. Under conditions and in systems where recovery was suppressed, inhibition was observed instead of stimulation. Thus, stimulation induced by the effect of cosmic radiation and other flight factors is similar to radiation stimulation. The similarity of these phenomena can be determined by a number of characteristics. Thus, space stimulation and radiation stimulation are variable, and they depend on environmental conditions to a great extent. Both processes are most pronounced in only the initial phases of growth. The stimulation effect greatly depends on the species and variety of plant, etc. But the size of the radiation dose which seeds receive while in space causes some doubt. It is known that a stimulating dose of radiation for the majority of cultivated plants is from 0.5 to 5 krad [15] and that the radiation dose of a month-long orbital flight is from 0.15 to 0.20 krad [10]. Even in the case of a solar flare, the integral radiation dose in a space ship rarely exceeds 0.3 krad [29], i.e., it does not reach the stimulating dose level for dry seeds. The facts are difficult to explain from the point of view of radiation biology, when the exposure of seeds of different radiation sensitivity in space causes the same number of chromosome irregularities in them, or when the seeds of radiation resistant species proved to be damaged more than radiation resistant plants [30]. It can be suggested that differences in the radiation sensitivity of plants on the earth and in space is due to a different composition of the ionizing radiation. However, experiments with an ionizing radiation source aboard a spacecraft have shown that the biological effect

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of the same dose and type of radiation in space flights is more strongly expressed in both warm-blooded and plant organisms [11, 42]. The increase in the radiation effect evidently is explained by the combined action of a number of space flight factors which reinforce the biological effect of an individual agent. One such factor is vibration.

Vibration is in that group of space flight factors which act on experimental objects during a short time interval. In particular, rocket engines cause vibration during ejection of the space vehicle into orbit and braking during the return to earth. The duration of the vibration overload of individual types of space vehicles differs little, and it rarely exceeds 20 min. However, the amplitude and frequency of the vibrations vary within broad limits, and they depend on the type of space vehicle, packing, shape and weight of the biological object, etc. The vibration load is particularly great during the launch of a space vehicle, and the vibration parameters change rapidly, but a more characteristic vibration overload is observed at frequencies of 100-500 Hz and amplitude of 0.4-4 mm [25, 49].

Based on the first experiments in space, scientists expressed the hypothesis that "dynamic factors" such as vibration play the leading part in the production of the biological effect in space flights [13]. Both a pronounced and a depressing effect of vibration was established [3, 14, 39]. However, no specific conclusions could be drawn about plant organisms, since an insufficient amount of experimental material has been accumulated so far. The biological effect of vibrations evidently greatly depends on a set of other factors /85 which operate inflight. In our experiments aboard the Kosmos-362 AES, we noted that the same radiation dose to lettuce seeds before the flight and after it has a different effect [21]. It can be proposed that the modifying factor in this case is the vibrations of space flight, which affect irradiated seeds in one case and unirradiated seeds in the other.

Laboratory experiments were conducted with lettuce seeds, a culture of isolated tomato roots and the model water plant Spirodella (Spirodella polirrhyza Schleid.). Harmonic vibrations of medium frequency (100 Hz, amplitude 0.6 mm) and ionizing radiation (1-3 krad dose of X-rays) were applied in different combinations. The experiments showed that the biological effect of vibration is extremely complex and basically depends on both the alternate combination with irradiation and on the physiological condition of the test subject during exposure. In the active physiological state, when the vibrations in themselves unfavorably affect the organism, the "vibration-irradiation" combination increases the radiation sensitivity of the organism. This regularity was established in both our laboratory tests with duckweed, where different phases of ontogenesis differ sharply according to physiological activity [22], and in the experiments of other authors [4, 40]. It is not excluded that vibration in itself has a favorable effect on actively growing plants in isolated cases. The "vibration-irradiation" combination then has a favorable effect on the organism [1, 23]. With respect to slightly active systems, such as dormant seeds, vibration frequently has a favorable effect. The "vibration-irradiation" combination which is applied in space flight conditions decreases radiation sensitivity of seeds and causes some stimulation in this case [23, 40].

In order to study this unique effect, we conducted experiments with the actively growing water plant duckweed. It turned out that the application of actinomycin D, in concentrations which depress RNA synthesis, substantially reduces the biological effect of vibration. On the other hand, depression of the genome of a plant cell, which occurs after the application of small doses of ionizing radiation according to the data of other authors [15, 46], activates RNA synthesis and thereby increases the effectiveness of vibration exposure. In experiments with seeds, in the combination "vibration-irradiation" exposure, this assumption is easily confirmed, since both factors emerge as synergists and cause some changes in the biological systems as a result [4, 22].

Thus, under space flight conditions, in which the primary vibration overload precedes cosmic radiation, dry seeds are subjected to the antagonistic effects of these two factors, and the unfavorable effect of vibration is not observed. However, no combination of these two factors under laboratory conditions provided a completely similar response of the plants to that induced by the entire set of space flight factors. Of this set, weightlessness is not reproducible under laboratory conditions.

Weightlessness acts on test subjects during the entire space flight, and it is a completely new factor of the environment which the living organisms of our planet have scarcely encountered. True, with respect to plants, weightlessness can be simulated by slow rotation of a horizontally placed plant around its xis, which is accom- /86 plished with a special clinostat apparatus. However, the studies of American scientists have shown that the clinostat removes the geotropic response of the plant, but it cannot completely simulate weightlessness [37, 40]. Study of the effect of weightlessness under flight conditions alone does not give a complete idea of the effect of this factor, since weightlessness occurs after exposure to the vibration overload and other factors which occur during launch of a space vehicle. It does not appear possible to create the state of weightlessness on the earth over a prolonged time. These procedural difficulties were overcome in the following manner. First, biological subjects were lifted into space in special vibration free spheres. Second, artificial gravity was produced aboard the AES, which simulated earth conditions during the flight. By comparing the biological effect of the test subjects in free flight and exposed to artificial gravity, a concept of the biological importance of weightlessness alone can be obtained, since the remaining flight factors do not play a part in this case. Such a unique experiment was conducted aboard the international Kosmos-782 AES, where part of the biological subjects was in a centrifuge which produced 1 g gravity during the flight. Together with colleagues of the Institute of Biomedical Problems, USSR Ministry of Public Health, we participated in study of the biological response of lettuce seeds exposed in this satellite [43]. The effect of weightlessness was studied by a number of biochemical, physiological and cytogenetic tests. It turned out that weightlessness had no noticeable effect on the free amino acid composition or soluble carbohydrate, starch or pigment content of 20-day old lettuce seedlings grown from the test seeds. The growth of the primary root and underground portion of the plant was somewhat increased [20], but the effect of weightlessness showed up particularly graphically in the chromosome rearrangements of the primary root of lettuce. The removal of weightlessness by artificial gravity significantly reduced the number of chromosome aberrations. The effect of weightlessness was expressed still more strongly in lettuce seeds which were irradiated with a small dose (1.5 krad) of gamma rays before the flight [26].

It is difficult to describe how the state of weightlessness affects the genetic structure of dry seeds. From the point of view of evolution, it can be proposed that higher plants are more sensitive to weightlessness than water plants. Therefore, the root system, as a characteristic organ of higher plants, is particularly sensitive to weightlessness [40], and chromosome disorders were detected in the meristem of the root. It is not excluded that synergism between the effect of weightlessness and ionizing radiation plays a large part in this case. This has been established in other organisms [48], but it should be noted that disruptions in a cell caused by flight factors have a tendency to disappear. Therefore, a complete description of the induced deviations can be obtained only in dynamic tests. Higher information content can be expected of the initial biological processes. One indicator of such primary processes is the phytochrome response in germinating light-sensitive seeds. It is based on the activation of the seed phytochrome by red light of a specific wavelength [45]. Depression of the phytochrome depends on the state of the cell genome, and it can reflect the effect of factors to which the light-sensitive seeds are exposed before germination [35]. The phytochrome response of lettuce seeds is extreme, which we determined in our preceding tests [23]. We were the first to use the phyto- /87
chrome response as an indicator of the initial biological processes

in space experiments [24], and it is advisable as a simple, extremely sensitive test. Thus, the phytochrome response of seeds which were exposed in the 1976 as probe sphere was depressed by 63% compared with laboratory control. Some depression was noted in subsequent growth of the primary rootlets. It was determined in experiments aboard the Kosmos-782 AES that, after removal of weightlessness, depression of the phytochrome response decreases, but it does not reach the laboratory control level [24].

There are data in the literature that flight duration has little effect on the biological effect in plants [2], i.e., space action of such constant factors as weightlessness and galàtic cosmic radiation is not cumulative in plant organisms. A more sensitive test object than dormant seeds evidently is required for a definitive explanation of the biological role of the individual factors. Even growing plants cannot give complete information on the effect of the flight factors, since the procedure of such experiments is very complicated.

The optimum solution would be exposure of activated seeds in space, the germination of which continues during the entire flight. In this state, the seeds are more sensitive to the effect of environmental factors. Together with colleagues of the biology department of the Latvian State University, we made an attempt to create a system in which lettuce seeds accumulated for a long time and preserved information on the environment. It was determined that, at reduced temperature, the seeds can be in an active, highly sensitive state for more than 20 days [33]. Unfortunately, this method still has not been tested under space conditions.

It should be noted in conclusion that, dry seeds have accumulated much information on the biological effect of space flight factors in the 15-year period of studies in space. In this review, only some of the most characteristic aspects of the effect of the individual factors and combinations of them have been discussed, and the area of our participation in these studies has been shown. It can be concluded from these data that the response of a plant organism to the effect of space flight factors is not pronounced. However, it should be emphasized that the material obtained is extremely contradictory and does not permit detailed, reliably substantiated predictions to be made. One of the causes of the nonuniformity of the results evidently is incomplete accounting for all the important biological factors. Thus, the conditions under which biological subjects are returned to earth have been studied little so far. The number of heavy space particles striking biological subjects has been extremely poorly studied. Changes in the magnetic and electromagnetic fields which, under extreme flight conditions can play an extremely important part, have been taken into account completely inadequately.

The continually increasing technical capabilities and the development of space flight complexes evidently will permit more complete accounting of flight factors to be obtained, and procedural questions of space plant growing to be solved as well. This will make it possible to determine the role of weightlessness and other space flight factors not only by the responses of air-dried seeds. Normally growing plants will also be included in the experiments.

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