GENETIC EFFECTS OF SPACE ENVIRONMENT

[Summary of Symposium at the XII International Congress of Genetics Held in Tokyo, 25 August 1968]

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WHEN Yuri Gagarin made his historic space flight in 1961, he took with him *Drosophila* flies to study the genetic effects of space environment. This was perhaps the first genetic experiment in space although genetic effects of space factors like the cosmic rays were investigated as early as in 1935. With the explosive development of space travel, both by manned and unmanned space crafts, the study of genetic effects of space environment like radiation and weightlessness and of space travel like acceleration, vibration, etc., have been under intensive study by both Russian and American geneticists. Their results, however, had not been subjected to a comparative analysis till the 25th August 1968, when both the groups met and presented accounts of their investigations at a Symposium held in Tokyo as a current meeting of the XII International Congress of Genetics. In view of the general interest of this topic, a summary of the principal results reported at the Symposium is presented in this paper.

Eleven papers were presented in three sessions into which the Symposium was divided based on the nature of the test material, *viz.*, (*i*) insects, (*ii*) cells, and (*iii*) plants. Four of the papers were presented by the Russian group, six by the American group and one by Dr. S. Kondo. The titles of the papers are listed at the end of this article. The three sessions were chaired by (1) Dr. R. F. Kimbal and Dr. E. N. Vaulina; (2) Academician N. P. Dubinin; (3) Dr. S. Fogel. The Symposium concluded with a final discussion under the Chairmanship of Dr. R. F. Kimbal and Dr. N. P. Dubinin.

In his opening paper, Academecian Dubinin[1]* referred to the challenges of space genetics, termed by him as 'cosmic genetics'. He highlighted the significant role of *Chlorella*, which might accompany man in his space flights both as a source of oxygen and of food, in future researches on space genetics. Among the basic genetical problems involved are, genetic selections to produce new forms adapted to the closed ecological system of space ships; investigations of evolutionary processes in populations of organisms aboard space ships to create artificial ecological systems, and genetic investigations of extra-terrestrial life forms in terms of molecular, cytological and genetic parameters.

Procedurally the techniques consisted in including the test organisms in space flights, both manned and unmanned, maintaining as far as possible strict ground controls, and testing the organisms after recovery back to earth. The various endpoints of these studies were, induction of recessive or dominant mutations, chromosomal aberrations of cell divisions, genetic recombination, etc. Irradiations during space flights were also carried out, in Biosatellite II launched by the American group, with the help of Sr^{85} on board, maintaining a control on the space craft itself through tungsten shielding, as well as on the ground. In the Russian flights, *Vostok-3* and *Vostok-4*, the astronauts crossed virgin *Drosophila* females with males during orbit. Pre- and post-flight treatments ranged from blood samplings from the astronauts to radiation treatments of test organisms and chemical mutagen treatments of *Crepis capillaris*. Although specifically not so designed, the biological systems under investigation provided a size gradient of the nuclei ranging from the biggest in *Tradescantia* to the smallest in bacteria. This permitted Kondo[9] to draw some theoretical conclusions. He inferred that space flight effects as noticed in the aberrations of cell divisions were the weakest in the micro-organisms with small nuclei and most pronounced in *Tradescantia* with the largest nuclei in the group. The total chromosomal mass in *Drosophila happened to be just* on the borderline of direct response to space effects, particularly weightlessness. Even in the micro-organisms, weightlessness can have an indirect effect through intercellular interactions when they are held in a liquid medium. A separation of the indirect effects can be

* Numbers refer to the papers listed at the end of this article.

achieved by adsorbing the cells on a solid surface or by holding them in a solid medium where free movement can be inhibited. This is yet to be tested.

The rest of the papers dealt with mainly an assessment of genetic effects of the space environment and the salient features of these can be summarised as follows :

(*a*) *Effects* of *Flight Duration.*—As flights are being planned with increasing time of orbiting or space travel, one of the primary areas that require elucidation is whether flight time is an important factor in aberration production. The results are not very conclusive in this regard. While prophage induction in bacteria[6] and aberrant mitoses in *Tradescantia*[12] increased with time in orbit, no correlation with the frequency of recessive lethals in *Drosophila*[2] with the length of flight could be established. Chromatid aberrations in the blood samples from the astronauts showed a tendency of increase with orbiting time.[8] But this was erratic and not consistent. It was concluded that flight time-action response was negative. The flight times considered in these studies ranged from 45 hours of the Biosatellite II to 8 days of the *Vostok* flights. Whether the negative results obtained in these studies would hold good for prolonged periods of weightlessness, a flight to Mars can be as long as 8 months, is an open issue.

(*b*) *Induction of Mutations.*—Active *Chlorella* cultures orbited for as long as 8 days aboard *Cosmos 110* earth satellite showed neither an increase nor a decrease of visible mutations after space flight.[10] The frequency of recessive lethal mutations on the sex chromosome of *Drosophila* (using two lines, high- and low-mutable) showed an increase in three out of seven experiments by the Russian group. A similar increased frequency was not noticed for the dominant lethals.[2] Likewise, in *Habrobracon* an increased frequency of recessive lethals was noticed.[5] But in the test material *Neurospora* included in the same mission as the *Habrobracon*, *viz.*, Biosatellite II there was no effect on the induction of recessive lethals concerning either the entire genome or specifically the *ad-3A* and *ad-3B* loci.[7] Similarly, for the somatic mutations for flower colour in *Tradescantia*, clone 02 heterozygous for this locus, no space flight effects were noticed.[12] It would thus appear that there are more instances of negative results than positive ones with reference to induction of mutations by space environments. In the case of the positive results obtained by the Russian group, the authors themselves feel that this could be explained as a function of the decreased fertility of the male flies due to factors non-specific to space flights.[2] It is interesting to note that in this study one of the genetic effects noticed on *Drosophila* after the space flight was the occurrence of crossing over in the germinal cells of the males in one of the three flights.

(*c*) *Effects on Genetic Recombination.*—The effects of space environment on genetic recombination was also negative in *Habrobracon*[5] as well as in *Drosophila*.[2] Increased recombination in *Drosophila* after space flight, noted by the Russian group, was attributed to temperature effects rather than space specific conditions. Recombination was found to be less frequent under near weightlessness resulting in the decreased probability of prophage P-22 detachment in *Salmonella typhimurium* (P-22)/P-22.[6] (This paper was scheduled but not read at the Symposium.)

(d) Synergism Between Radiation and Space Environment.—Several studies were devoted to the combined effects of artificial radiation and space environment. The radiation treatments were given as pre-, or post-flight treatments with or without orbital irradiation. In *Drosophila* there was an increase of sex-linked recessive lethals amongst the daughters of irradiated flies during the flight specimens compared with the irradiated ground controls.[3] In the case of *dumpy wing* the increase was nearly four-fold but was estimated to be just significant when allowance was made for the non-normalities of distribution at frequencies as low as 14/20,582 and 4/24,838 for the orbital and control group respectively. Likewise, the frequency of loss of markers from the Y chromosomes among the sons of males irradiated in the pupal stage was significantly higher in the orbited group than in the ground controls. The frequency of translocations between the Y chromosome and the second pair of autosomes among the sons of males pre-flight irradiated with 4,000 *r* units of X-rays was significantly *less* in the during-the-flight irradiated group. Radiation obviously interacts with weightlessness resulting in cell lethal chromosome breakages, loss or exchanges of chromosome elements and sex-linked recessive lethal mutants to a greater degree than irradiation given on earth.

In *Neurospora*, on the other hand, no effects of space flights in combination with gamma-radiation was observed,[7] when the end-points of the study were the survival of heterokaryotic conidia or induction of recessive lethal mutations. In the case of human blood samples there was a slight increase in the frequency of single break chromosomal aberrations but not in that of the multiple break aberrations in the in-flight irradiated group when compared to the control, in the Gemini III flight. However, these results could not be reproduced in the subsequent Gemini XI flight. Presumably the earlier results are to be attributable to sampling errors.[8] In *Tradescantia* radiation induced somatic mutation rates were unaffected by space flight.[12] But increased pollen abortion, stamen hair stunting and chromosome exchanges indicate increased degree of injury by gamma-irradiation during space flight over the earth-based control. This applies to the sensitive stages of both mitosis and meiosis. There appears to be no general trend of synergistic action on radiation effects by space conditions even within the same test organism. Thus, while space factors enhanced the frequency of dominant and recessive lethals when metaphase I oocytes of *Habrobracon* were irradiated almost by a factor 2, transitional oogonia irradiated during flight showed much less damage than ground-based irradiation suggesting even radiation resistance. The maximum enhancement of radiation effects was in the induction of translocations between 2 and 3, and 3 and 4, in *Drosophila* spermatogonia.[4]

The only conclusion that these contrasting results permit appears to be that the enhancing of radiation damage by space factors does not by itself constitute a specific hazard for space flight.

(*e*) *Cytological Effects.*—In higher plants as well as in human cells chromosome aberrations were unaffected by space factors (with or without concurrent irradiation). Exposure of seeds of *Crepis capillaris* to space factors enhanced their sensitivity to ethylenimine treatments after their return to earth,[11] a significant increase in the chromosome rearrangements being found. Disturbances in cell division or mitotic disorders in *Tradescantia* or primary non-disjunction in *Drosophila* were the more pronounced effects of space factors than chromosome aberrations. In *Tradescantia* the spindle mechanism was disturbed both in the microspores and in root-tip cells. These results point to the possibility that a living cell might be affected by space conditions more through an indirect action on the other cellular organelles and processes than through a direct effect on the nucleus.

(*f*) *Other Effects.*—Amongst the many other effects reported upon, the longer life-span of *Habrobracon* females exposed to space conditions than the ground-based controls[5] and disturbances in the sporulation pattern in *Chlorella*[10] are of interest.

In conclusion it may be pointed out that notwithstanding variations in flight factors, like orbiting altitude, acceleration, vibration, closeness to zero gravity, the diversities of test organisms and the differences in their physiological stages of exposure to space conditions, and the divergences in the genetic end-points considered, the similarities in the conclusions drawn by the American and the Russian groups are indeed very striking While the genetic effects of space environment are bound to be probed deeper and deeper with every advancement in space flight technology, to the earth-bound geneticists these results will stimulate thinking on the significance of the role that earth's gravitational force has played in the regulation of cell division, differentiation and other genetic mechanisms in the evolution of higher forms of plants and animals on *terra firma*.

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1. Dubinin, N. P., Gazenko, O. G., Vaulina, E. N. and Parfenov, G. P., "Challenges of Space Genetics".
2. Glembotsky, Ya. L and Parfenov, G. P., "Genetic Investigations with *Drosophila* in outer space" (Presented by an interpreter).
3. Browning, L. S. and Altenberg, E., "Effects of the space environment on radiation induced damage in the reproductive cells of pupae and adult *Drosophila*".
4. Oster, I. I., "Genetic effects of zero gravity and radiation".
5. von Borstel, R. C., Smith, R. H., Grosch, D. S., Whiting, A. R., Slater, J. V., Buckhold, B. and Tobias, C. A., "Experiments with *Habrobracon* and *Tribolium* on Biosatellite II".
6. Mattoni, R. H. T., "Influence of space flight and radiation on induction of prophage P-22 in *Salmonella typhimurium*" (This paper was not presented).

7. de Serres, F. J. and Webber, B. B., "Effects of weightlessness on radiation-induced inactivation and mutation induction in *Neurospora crassa*".
8. Bender, M. A., Gooch, P. C. and Kondo, S., "Human blood experiments in the Gemini Series".
9. Kondo, S., "Possibility and impossibility for genetic effects of weightlessness".
10. Vaulina, E. N. and Anikeeva, I. D., "Space effects in *Chlorella*".
11. Dubinina, L. G. and Chernikova, O. P., "Space effects in *Cretis capillaris* seeds".
12. Sparrow, A. H., Schairer, L. A. and Mamuthu, K.M., "Genetic and cytological studies of *Tradescantia* irradiated during orbital flight" (This paper was presented by F. J. de Serres).

ASHING PROCEDURES FOR BIOMATERIAL*

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VARIOUS methods have been adopted for the destruction of organic matter in biomaterial before estimating the mineral content. Usually, biomaterial are ashed either by "dry ashing" or by "wet digestion" methods. The former procedure is most commonly followed in which mineralisation of organic material is achieved by destroying the samples at different definite temperatures.[1] In the latter method, oxidation is carried out by an oxidising agent in solution.[2-4] However, varying degree of losses of elements have been reported during ashing. Methods so far used for the estimation of the loss of trace elements during ashing procedures involved the direct addition of radiotracers to the excised tissues.[1-4] Such addition, however, does not take into account the possible elemental losses from the volatile organometallic complexes in the biological system during the decomposition of tissues. In the present studies, therefore, an attempt has been made to study the loss of elements incorporated in the biological system. This was achieved, by injecting radiotracers into the living system and allowing the animal to live for stipulated period before sacrifice, and followed by various ashing procedures. This laboratory has been studying the radioecology of an arcid clam *Anadara granosa* (Linn.) for the past few years.[5,6] This clam was therefore selected to study the loss of trace elements during ashing procedures.

EXPERIMENTAL

The radioisotopes of high specific activity used in the present study with half-lives and chemical forms are given in Table I. Freshly collected clams were washed in running sea-water and acclimatised at 22° C. Isotopes (about 10-15 mμC/animal) were then injected in the pedal sinus of the bivalve and maintained in filtered sea-water. After about 48–96 hours, these were dissected, the tissue was transferred (8–12 gm.) into a plastic counting vial and the activity was measured by gamma spectroscopy using a well-type NaI (Tl) crystal.

TABLE I

List of radioisotopes injected with the half-lives and chemical forms

Isotopes	Half-life	Gamma energy (meV)	Chemical form used
Iron-59 ..	46 days	1·097	Ferric chloride (4 C/gram)
Strontium-85 ..	65 „	0·513	Strontium nitrate (0·1 mc Sr-89/gram Sr)
Cobalt-58 ..	71 „	0·810	Cobalt (II) chloride (carrier free)
Zinc-65 ..	245 „	1·110	Zinc chloride (5 mc/gram Zn)
Cerium 144 ..	290 „	0·134	Cerium (III) chloride (carrier free)
Manganese-54	291 „	0·834	Manganese (II) chloride (carrier free)
Cesium-137 ..	28 years	0·660	Cesium chloride (carrier free)

(*a*) *Dry Ashing.*—The tissue from the plastic vial was quantitatively transferred to a silica crucible and dehydrated under an infrared lamp. Ashing was carried out in a muffle furnace at 400° C. and 700° C. for about 24 hours. The ash samples were dissolved in dilute hydrochloric acid and transferred to the counting vial and counted maintaining the same geometry as of the wet tissue.

(*b*) *Wet Ashing.*—The wet ashing was carried out using oxidising liquids, namely, HNO_3, combustion mixture (H_2SO_4 : $HClO_4$: HNO_3) and mixture of HNO_3 : H_2O_2 (Table II).[7-11] Tissue

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