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16 May 1963

Corn Seeds Affected by Heavy Cosmic Ray Particles

Abstract. *Corn seeds of a special genetic stock were recovered from two satellite flights and the plants grown from them were examined for abnormalities. Some evidence for a slight increase in chromosomal deletions was observed, which was predicted from the flux of heavy cosmic ray primary particles. Nothing unexpected was observed.*

The ionizing radiations which exist at altitudes greater than 120 km, where the earth's atmosphere is very sparse or nonexistent, have been extensively studied in recent years with the aid of balloons and satellites. The biological effect of these radiations has been predicted from the physical measurements alone, but it seems desirable to test these predictions with appropriate live material.

The present experiments employed corn seeds as the test material. The seeds were flown in satellites and returned to the laboratory, where they were germinated and grown. Certain of the early leaves were examined for abnormalities which would indicate radiation damage to the embryo. If radiation causes genetic damage in one or more individual cells of a leaf primordium of the seed embryo, then for each mutated cell a change can be observed at a corresponding point in the leaf of the plant.

The radiations can be divided into three categories, as follows. (i) Electromagnetic radiations such as x- or gamma rays which would be expected to have a negligible effect on the seeds at the radiation levels encountered. (ii) Electron and proton radiations which comprise by far the most abundant type of radiation and would be expected to cause genetic damage in individual scattered cells of the seed embryos. Readings from ionization chambers in the satellites, together with experience gained from previous exposures to known radiation sources, permit an accurate estimate to be made of the frequency of leaf sectors to be expected from this source. (iii) Heavy cosmic ray particles, consisting of stripped atomic nuclei having masses as high as iron and traveling at very high speeds, may produce a very broad path of dense ionization as they enter matter. As the particle slows down the track increases in ionization density, and just before its end it becomes very broad and dense. This section, known as a thindown, may be as much as 25 μ in diameter in tissue and several millimeters long. Since the particles are traveling very fast in outer space, almost none of them would be expected to be slowed down in a small object, like a package of seeds, sufficiently to form a thindown. However, behind rather thick shielding or when the particles have penetrated the atmosphere some distance, thindowns should be encountered rather frequently. Schaefer (*1*) has shown the maximum number to occur at an altitude of about 40 km and to decrease sharply at higher altitudes, reaching zero in an unshielded situation in outer space beyond about 80 km.

These high-energy heavy particles cannot be produced in the laboratory, so there has been no direct biological experience with them. Thus the chief interest in this experiment was with this type of radiation, and corn seeds were chosen as the test object because they would be expected to respond to it in an observable way.

In general, the biological damage produced is proportional to the ionization produced, and for the heavy particles this ionization is concentrated in very small volumes, except for the thindown portion of the track. The diameter of this part of the track may be wide enough to hit several cells of the corn embryo, and within its core the ionization would be very dense. If such a track went through an embryo, one would expect damage to the primordia of all leaves through which it passed. The plant grown from such a seed might show damaged areas in several leaves, and from the positions of these one should be able to estimate the course and extent of original damage to the embryo as it was traversed by the particle. The very-high-speed heavy particles encountered would be expected to cause occasional damaged cells in the seeds which would show as mutant streaks in mature leaves. It was this speculation that the experiment was designed to verify.

The corn seeds used in these experiments have embryos in which six leaves or leaf primordia are present in various stages of development. Observations on leaves 3 and 4 were used in these experiments to obtain quantitative data on genetic damage. These leaves are most easily scored because of the size and frequency of mutant sectors produced, which, in turn, are due to the particular combination of numbers of target cells in the embryonic initials and the amount of cell division and expansion that occurs in subsequent growth. The seeds employed were of a genotype that is heterozygous for alleles controlling green (Yg_2-dominant) versus yellow-green (yg_2-recessive) color of the leaf. The larger the dose of radiation delivered to one of the cells of an embryonic leaf, the greater is the probability that the cell will undergo chromosome breakage, and the higher is the frequency of loss of the allele (Yg_2) responsible for green color. As a consequence of such a loss, this altered cell and all its progeny will fail to form the fully green chlorophyll of normal leaf cells. Thus, a single "mutation" in an embryonic leaf cell in this stock will show up in the growing plant as a yellow-green streak or sector in the mature leaf (*2*). A microbeam of deuterons from the Brookhaven cyclotron has been developed as a tool for simulating the biological effects of the thindown particles (*3*) and the effects of these beams on this genetic stock of corn have been described (*4*) and these results have been used to predict the appearance of a thindown hit in this material.

Seeds were flown and successfully retrieved from two satellites: Discoverer 32 launched on 13 September 1961, and a satellite launched in midsummer 1962 (*5*). These satellites were in polar orbit with an average altitude of about 280 km for 27.3 and 49.6 hours, respectively. In addition to the seeds, the second flight had some nuclear track plates packed in the corn.

After recovery, the corn was grown in a controlled environment room, along with control samples which had been sent to Vandenberg Air Force Base with the flight samples but not flown. The results from coded scoring of the frequency of yg_2 streaks on leaves 3 and 4 are presented in Table 1. An analysis of variance of the data from the first flight, utilizing the approximate proportionality of the number of leaves scored, gave no evidence for a significant increase in the frequency of streaks in the corn recovered from the flight as compared to the controls. An analysis of variance of the data from the second flight, based on the method of weighted squares of means, revealed a significant increase in sector frequency for flight seed scored for leaf 4 but not for leaf 3. On the other hand, it can be seen from the data that, in the ten comparisons made (five seed lots, two leaves each) for the two flights combined, in eight of these the sector frequency for the flight seed was greater than for the controls, and in one it was the same. Friedman's (*6*) rank sum test applied to the pooled data gives evidence that there is significantly more sectoring, but only at the 5-percent level ($\chi^2_1 = 4.9$), in plants grown from the flight seed. The conclusion reached from this analysis is that there was little, if any, increase in sector frequency due to the flights.

Table 1. Frequency of yg_2 sectors.

Seed lot	Leaf 3				Leaf 4			
	Control		Flight		Control		Flight	
	No. of leaves	yg_2 per leaf	No. of leaves	yg_2 per leaf	No. of leaves	yg_2 per leaf	No. of leaves	yg_2 per leaf
				First flight				
A	89	0.034	90	0.056	89	0.022	91	0.022
B	94	.085	92	.120	94	.043	90	.067
$\overline{m}$		.060		.088		.033		.044
				Second flight				
A	93	.086	206	.116	93	.032	206	.048
B	86	.081	187	.070	86	.035	187	.054
C	121	.050	162	.056	121	.016	162	.037
$\overline{m}$		.070		.083		.027		.047

An exceptional yg_2 sector frequency observed in one plant may have been due to a thindown particle hit on Discoverer 32. In one-half of leaf 3 there were six separate yg_2 streaks, in leaf 4 there were two such streaks, and one also appeared in leaf 5. These leaves overlap in the embryo so that this pattern of hits may indicate a single major thindown traversal. With this possible exception there was no conclusive evidence of more radiation damage in the flight samples than in the controls.

In addition to the data taken on the frequency of yg_2 streaks, observations were also made on the occurrence of cut or notched leaves, aborted shoot apexes, and files of dead leaf cells in both the genetic stock and a commercial hybrid corn that was used as packing in the first flight. These abnormalities were found to be no more abundant in the flight samples than in the controls.

The numbers of yg_2 sectors to be expected were estimated in several ways. The U.S. Air Force included ionization chambers on these two flights, and they recorded about 15 mrad and 3 rad, respectively. This ionization would be due almost entirely to electrons, protons, and x-rays. This amount of radiation would cause a negligible amount of radiation damage in these seeds. Thus any damage observed must have been due to heavier particles.

The flight film and control plates, were developed along with an identical plate which had been exposed to 44-mev alpha particles as a reference for relatively heavy particle tracks. The plates were carefully scanned for heavy particle tracks, and any tracks heavier than the alpha tracks were scored. In all, 64 cm^2 of emulsion were scanned on both flight and control plates and the flight plates showed a track frequency of two tracks per square centimeter for the 49.6-hour flight. None was found on the controls. This agrees well with results obtained by Hewitt and Campbell (*7*). However, none of these tracks was more than about twice the ionization density of an alpha track, so that all were from particles traveling at such velocities that they did not produce tracks heavy enough to be classed as thindowns. The one apparent thindown hit observed in one seed from the first flight was probably a real effect, since there was certainly a finite probability of receiving such a hit.

These emulsions gave information only on the very heavy ionization tracks, that is, those above alpha track ion densities. It is possible to compute from the work of Schaefer (*1*) the total number of heavy particles passing through the samples; this amounts to about 380 and 670 per square centimeter, respectively for the two flights. There would also be some slow protons which would cause dense ionization tracks, but it is very difficult to estimate their numbers. Calibration measurements have indicated that a low-energy proton flux having about the same ionization density as the very energetic heavy particles will produce about one streak on leaf 3 per seed for a flux of 10^6 protons per square centimeter. For the first flight, on this basis, one might expect for the 182 seeds no significant increase in mutant streaks, but for the second flight, with 555 seeds, there might be an increase between 0.001 and 0.01 streak per leaf. This is at best a rough approximation, but is accurate enough to explain the apparent slight increase in streaks found in this experiment. It clearly indicates that if the very heavy particles are traveling at very high velocities, they can pass through living cells and produce little damage. This is in accord with radiobiological expectations.

Particles producing ionization tracks such as these are known to be very effective relative to sparsely ionizing tracks in producing chromosome breaks in corn seeds, and this is the reason one would expect a slight increase in streaking even though the ionization chamber readings were very low.

The real purpose of this experiment was to verify the predicted radiobiological effect of the heavy cosmic ray primary particles, and to test whether any unexpected biological phenomena existed in a satellite environment. Within the limits of this biological system, nothing unexpected was found (*8*).

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Radioprotection by Pressor Amidines

Abstract. *In the mouse, radioprotection is not always associated with the effect of hypertensive amidines and related amines. The protection resulting from this group of agents follows the pharmacological reduction of intercellular oxygen tension.*

After the observation that simple *S*-alkyl isothiuronium salts decrease radiosensitivity, Ashwood-Smith (*1*) tested some of its homologs in an attempt to relate structure to radioprotective action and to discover more promising agents. He found that activity diminishes rapidly as the *S*-alkyl substituent is lengthened beyond three carbon atoms. It is interesting that Fastier (*2*), in his excellent review of the structure-activity relationships of amidines, describes a loss of pressor action for *S*-alkyl isothiuroniums with alkyl substituent longer than three carbon atoms. The possible correlation of chemical structure, pressor activity, and radioprotection by these amidine derivatives led to a study of the effects of pressor amidines and pharmacologically related amines on the radiosensitivity of mice.

Table 1. Thirty-day survival data of mice receiving single doses of related pressor amines and amidines before irradiation to lethal doses of Co^{60} (1000 r).

Intraperitoneal administration		Animals (No.)	Survival (%)
Dose (mg/kg)	Time (min)		
Controls			
		290	0
2-Methylpseudo urea			
500	15	20	50
Methyl guanidine			
150	5	20	5
2-Amino pyridine			
25	15	30	0
4-Amino pyridine			
3	15	15	0
n-*Pentylamine*			
50	15	10	0
n-*Hexylamine*			
40	15	10	0
S-*ethyl isothiuronium*			
150	30	40	98
150	15	80	90
75	15	30	90
20	15	20	60
*Papaverine · HCl**			
325	30	10	10
Papaverine • HCl, plus* S-*ethyl isothiuronium*			
325	30		
150	15	26	40
Hypoxia†			
		42	5
Hypoxia, plus S-*ethyl isothiuronium†*			
150	15	20	5

* Subcutaneous administration. † Irradiated with 2200 r Co^{60}.

Young female mice (Bagg Swiss), weighing 20 to 25 g, were used. Ten control mice were irradiated simultaneously with each treated group and thereafter both groups were housed jointly. The radiation was done in a specially designed cobalt-60 irradiator which contained about 1200 curies of cobalt-60, half above and half below the radiation chamber. The mice were exposed in a plexiglass box which rotated through a flat radiation field of about 100 r/min. In the experiment with hypoxia, two treated and two control mice were irradiated simultaneously in a cobalt-60 Gammacell-220 (*3*) at about 1800 r/min. The irradiation chamber was gassed before and during exposure with a mixture of 5 percent oxygen and 95 percent nitrogen.

Each of the chemicals tested is known to increase blood pressure (*2*), but only two of these offered significant protection against lethal radiation. The survival data in Table 1 indicate that radioprotection by amidines is not directly associated with their pressor activity. In an attempt to explain this disparity, additional investigations were conducted with *S*-ethyl isothiuronium as a test compound.

The results in Table 1 show that *S*-ethyl isothiuronium is radioprotective when used over a wide dose range and for a considerable period of time. Also, papaverine, a known pharmacological antagonist (*2*) significantly reduced the protective effect of a massive dose of *S*-ethyl isothiuronium. Other agents—reserpine, atropine, phenergan, and dibenzyline—had no influence on *S*-ethyl isothiuronium action. The favorable therapeutic ratio and the response to a specific antagonist are parallel to actions established for serotonin (*4*), which is thought to decrease radiosensitivity through oxygen-dependent pathways. A similar mechanism may explain the action of *S*-ethyl isothiuronium since our data show that it fails to increase the radioprotection afforded mice by the optimal reduction of intercellular oxygen.

The experimental results suggest that pressor amidines offer radioprotective activity through a pharmacological mechanism which leads to a lowered oxygen tension of radiosensitive tissues.

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Glycogen Deposition in the Liver Induced by Cortisone: Dependence on Enzyme Synthesis

Abstract. *The deposition of liver glycogen in starved rats given a single dose of cortisone is inhibited by puromycin and actinomycin. The former agent interferes with induced enzyme formation in general, and the latter with the cortisone-induced rise in liver enzyme levels. The results suggest that the regulatory effect of cortisone on carbohydrate metabolism may be brought about by its action on the cellular concentration of certain enzyme proteins.*

Adrenocortical hormones, which influence the rate of certain metabolic processes in vivo, do not appear to act as simple inhibitors or activators of enzymic reactions in vitro. Therefore, Knox, Auerbach, and Lin (*1*) suggested that hormone action may be brought about by changes in the actual concentration of the protein moiety of specific enzyme systems. The dependence on enzyme synthesis of the acute stimulation of glycogen deposition by cortisone in the liver of starved rats has now been tested.

Recent data suggest that the rise of enzyme activity induced by cortisone reflects an increase in the rate of *de novo* enzyme synthesis. The accumulation of liver tyrosine transaminase (*2*), glutamic-alanine transaminase (*3*), and tryptophan pyrrolase (*4*) has been measured immunochemically. Correspondingly, the administration of an inhibitor