Technical Papers

Effect of Gamma Irradiation on the Ascorbic Acid Content of Green Plants[1]

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During the summer of 1952 a preliminary investigation of the effect of gamma irradiation on the ascorbic acid content of plants was undertaken. As it is, at this time, impossible for the writer to continue with this work the results of these experiments are presented now.

The effect of continuous irradiation on ascorbic acid content was studied by growing the plants near a source of constant gamma rays. The plants were irradiated continuously, except for short periods of time during the day when it was necessary to enter the gamma field to collect material. Snapdragon plants, varieties rose queen and afterglow growing in pots, were placed 1.3 meters from a 300-curie Co^{60} source. The gamma irradiation at this distance as measured by victoreen r-meter cobalt chambers was found to be approximately 5000 r/day. The control plants received no gamma irradiation. Total ascorbic acid was determined periodically by the method of Roe and Oesterling (*1*). The effect of chronic gamma irradiation on the ascorbic acid level in these plants is shown in Table 1.

TABLE 1

EFFECT OF CHRONIC GAMMA IRRADIATION ON THE ASCORBIC ACID CONTENT OF SNAPDRAGON PLANTS

No. of days in gamma field	Ascorbic acid level in irradiated plants expressed as percentage increase over controls	
	var. rose queen	var. afterglow
2	+ 4.5	−13.8
3	+ 5.7	+10.7
5	+24.5	+41.1
7	+12.4	+47.4
9	+69.4	+51.4

As can be noted from the data there was a rise in ascorbic acid content of the plants with time for a period after the start of irradiation. This rise in ascorbate was noted before there was any visible damage to the plants. The plants did show radiation damage after a longer period of time. A rise in ascorbic acid content was also noted after an acute dosage of x-rays. The relation of x-ray dosage to increase in ascorbic acid content is given in Table 2. In this experiment Biloxi soybean plants were exposed to various dosages of x-rays which were administered at the rate of 1000 r/min. The x-ray machine was operated at 250 kv and 30 ma, with a filter of 1 mm of aluminum. After irradiation the exposed plants were placed in the greenhouse with the control plants.

TABLE 2

EFFECT OF VARIOUS DOSAGES OF X-RAYS ON THE ASCORBIC ACID CONTENT OF SOYBEAN PLANTS

No. of days after irradiation	Ascorbic acid content in irradiated plants expressed as percentage increase over controls		
	1000 r	4000 r	16000 r
2	−10.1	−10.1	− 4.1
3	− 4.5	+19.5	+ 9.1
5	+ 8.3	+18.6	+33.8
10	+ 9.5	+12.9	+28.4

From these and other preliminary experiments it would appear as though the immediate effect of irradiation is a drop in the ascorbate level of the plants. This is followed a few days later by a rise in the ascorbate level, followed some time later by a second decrease in ascorbic acid content. The cause of these increases in ascorbate upon irradiation has not been further investigated. The increase might possibly be due to a decrease in activity of enzymes bringing about the oxidation of ascorbic acid. Weber and Gordon (*2*) were able to show a significant increase in indoleacetaldehyde following x-irradiation and at the same time a decrease in the ability of an enzyme to convert indoleacetaldehyde to indoleacetic acid.

Ascorbic acid is one of the naturally occurring compounds in biological systems that appears to offer some protection against irradiation. Gordon and Weber (*3*) showed that ascorbic acid protected solutions of indoleacetic acid from irradiation. Giri (*4*) showed that isolated enzyme systems were protected by ascorbic acid, and Caffaratti (*5*) was able to show that injection of ascorbic acid into the blood stream of rats protected these animals from whole body irradiation. However, the rise in ascorbic acid content following irradiation of plants as noted in these experiments did not further protect these plants against irradiation damage. An interesting phenomenon noted during the course of these experiments, however, was the apparent correlation between the initial ascorbic acid content and the relative resistance of a plant to irradiation. This was especially noticeable in that part of the work where plants were grown under constant gamma irradiation (*6*). Table 3 shows the relative resistance of a plant to irradiation and the normal ascorbic acid content of that plant.

Table 3 suggests that there may be some correlation between the normal ascorbic acid content of a plant and the sensitivity of that plant to irradiation. Many more species of plants should be examined, however,

[1] This work was carried out at Brookhaven National Laboratory, Upton, L. I., N. Y., while the author was research collaborator.

TABLE 3

ASCORBIC ACID CONTENT AND THE RELATIVE RESISTANCE TO IRRADIATION
(In mg/100 g fresh wt)

Species	Resistant	Moderately resistant	Low resistance
Cabbage	200–300		
Gladiolus	300–400		
Soybean		120–150	
Snapdragon		100–120	
Cosmos sulfureus			90–100
Nicotiana rustica			50– 60
Xanthium spp.			70–100
Hyoscyamus niger			30– 40

before a definite conclusion can be safely drawn. This ability to resist irradiation depends on the plant's normal ascorbic acid content and not on the increase in ascorbate upon irradiation. From these experiments it appears that ascorbic acid may offer a protecting action against irradiation in plants.

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The Ultraviolet Microscopy of the Living Cell's Response to Lethal X-Radiation[1]

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The development of the Polaroid color translating ultraviolet microscope by Land and co-workers (*1*) has brought to biology and medicine a tool capable of following the nucleoprotein changes in living cells subjected to lethal doses of x-radiation. This paper presents the data obtained when tissue cultures of Walker rat carcinoma 256 cells are given a lethal dose of x-radiation and then photographed with the Polaroid color translating ultraviolet microscope for intervals up to 120 hr.[3]

The technical aspects of the Polaroid color translating ultraviolet microscope have been published elsewhere and will not be discussed here (*1–3*). Such data as those given in the following paragraph, however, are pertinent to the experiments reported.

U-V source: High-pressure AH-6 mercury arc operated at 600 volts

Wavelengths: 281 mμ, blue filter; 261 mμ, green filter; 250 mμ, red filter

Band-width at half height: 5.3 mμ

Objective: Polaroid-Grey No. V, N. A. 0.72

Condenser: Polaroid-Grey No. V, N. A. 0.72

Diameter of specimen area illuminated: 75 μ

Original magnification to film: 210×

Film type: Kodak Spectrum Analysis #1

Rapidly processed with D-8 developer diluted 2 : 1, at 90° F for 7 seconds

Specular gamma: 1.70

All photographs reported in this experiment gave a background density of approximately 2.20 density units.

All x-radiation was given by a General Electric Maximar therapy unit, 200 kv, 10 ma, inherent filtration 3 mm aluminum, target distance 20 cm.

The tissue culture preparations were made from a suspension of Walker rat carcinoma 256 cells in a nutrient medium composed of 10% embryo extract, 50% human ascitic fluid from a cirrhotic patient, and 40% Hank's balanced salt solution. The suspension was made by excising small pieces of tumor from subcutaneous transplants, and then grinding the fragments lightly with several applicator sticks while they were suspended in medium. One drop of this suspension was then placed on half of a Vycor cover slip, the other half of the cover slip was placed on top, and the excess fluid was removed from the edges. The two halves of the Vycor cover slips were then placed in roller bottle flasks as flying cover slips, with sufficient medium added just to cover them. In this type preparation the surface tension of the cell suspension is sufficient to prevent the cover slips from separating, while medium is permitted to diffuse slowly between. In this fashion the cells maintain a continuous population which covers the area between the cover slips. One-half of the medium was renewed every 3 days. The Slonaker rats in which the tumor was carried subcutaneously were treated with penicillin, and penicillin was added to all cultures. The cell suspensions were made from tumors which were from 10 to 15 days old. All flasks were kept in the incubator at 37.5° C.

It was determined that approximately 90% of the cells were carcinoma cells by means of supravital stains with Janus green and neutral red, as well as such fixed stains as Wright's and Giemsa's. The remainder of the cells were macrophages.

In all cases the tumor was cultured the day previous to x-radiation, and control cultures made and carried under identical circumstances. The day of photography the cultures were washed with balanced salt solution by substituting it for the medium for 30 min. The double cover slips were then mounted on

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[3] The ultraviolet photographs were taken on the Polaroid color translating ultraviolet microscope in the Research Division of the Polaroid Corporation, Cambridge, Mass.