A COMPARISON OF THE EFFECTS OF X-RAYS AND THERMAL NEUTRONS ON DORMANT SEEDS OF BARLEY*

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Introduction.—The present investigation was initiated to compare the effects of irradiating dormant seeds of barley with x-rays and thermal neutrons on seedling height and chromosomal aberration frequencies. The results presented here show that there are differences in the response of dormant seeds of barley to x-rays and thermal neutrons. It is hoped that these data may be of some significance in interpreting the mechanism of the effect of ionizing radiations on biological systems.

No attempt will be made to review the literature relevant to the effects of x-rays and thermal neutrons on living organisms. The reader is referred to Lea[1] for a discussion of x-ray effects. The excellent paper by Conger and Giles[2], and papers by Frolik and Morris,[3, 4] Rasch,[5] Schmidt and Frolik[6] and Zirkle[7] constitute most of the available literature on the effects of thermal neutron radiation of living organisms.

Material and Methods.—In the present investigation samples of 250 dormant seeds of Himalaya barley, which had been kept in a humidity-controlled desiccator for at least three weeks prior to treatment, were subjected to the following dosages of irradiations at the Brookhaven National Laboratory: x-rays—5000 r; 10,000 r; 15,000 r; and 20,000 r; and thermal neutrons—3, 6, 9 and 12 hrs. at 4.65×10^8 N_{th}/cm.^{2}/sec.

For x-raying, the seeds were placed in a single layer in a Pyrex Petri dish located 21 cm. from the tube. The radiation was filtered with 1 mm. of aluminum and was administered at 250 kvp. and 30 ma. Under these conditions the seeds received approximately 1200 r per minute as determined with a Victoreen intergron.

For treatment with thermal neutrons the seeds were placed in a single layer in a leucite container and exposed in the thermal column of the nuclear reactor. Flux calculations were determined with gold foils exposed with the seed.

After irradiation, seeds from each treatment along with control samples were handled as follows: (1) 150 seeds of each treatment were planted in randomized blocks in greenhouse flats for observations on seeding height, and (2) 100 seeds were germinated in Petri dishes and root tips removed for cytological analyses.

Individual seedling height measurements were taken at 14 days after planting on the material grown in the greenhouse. The data obtained were subjected to an analysis of variance.

For the cytological analysis root tips were removed from germinating seedlings at the time of the first cycle of cell divisions. They were fixed and stored in Carnoy's 6:3:1 (alcohol:chloroform:acetic acid) solution. Anaphase bridge and fragment frequencies were determined on these samples, using the aceto carmine smear technique.

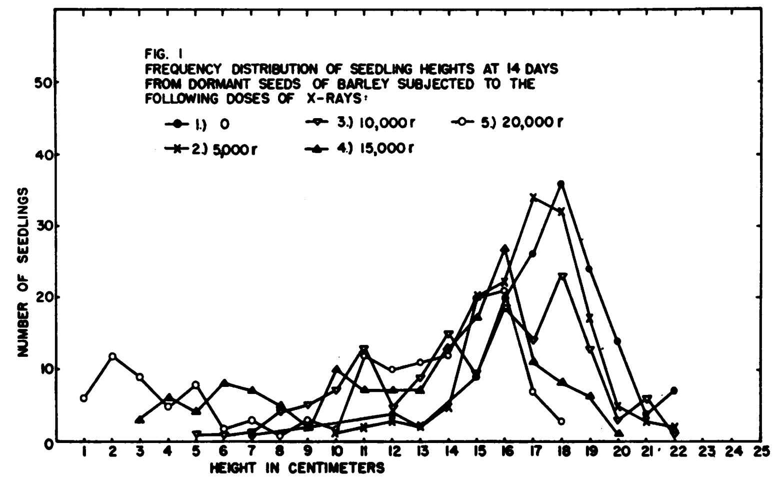

FIGURE 1

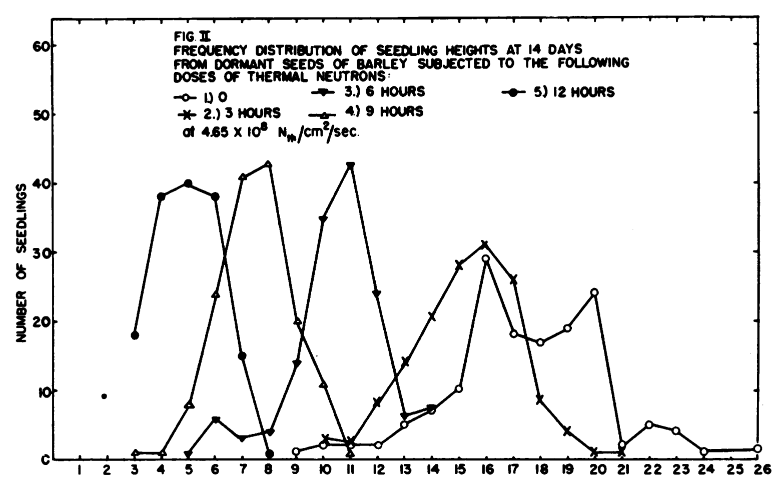

FIGURE 2

Experimental Results.—A striking difference was observed in the effects of x-rays and thermal neutrons on the height of seedlings at 14 days. With x-rays, at doses of 10,000 r and above, there was a considerably greater range in individual seedling heights than there was with thermal neutrons. Furthermore, the magnitude of the difference increased with the increase in dosage of x-rays, so that at a dosage of 20,000 r individual seedling heights ranged from 1 to 18 cm. This has added significance when it is considered that the average height for the untreated control was only 17.7 cm. The frequency distributions of the seedling heights from seeds treated with x-radiation are depicted graphically in figure 1. According to an analysis of variance differences between treatments were significant.

When seeds were irradiated with thermal neutrons, individual seedling heights at any given dosage were no more variable than were the controls. Frequency distribution curves for the different dosages of thermal neutrons are presented in figure 2.

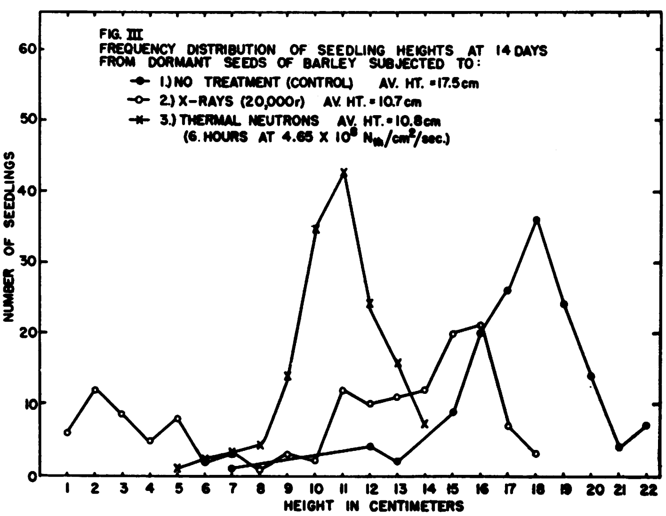

FIGURE 3

In figure 3 frequency distributions are shown graphically from data which include a control along with a dosage of x-rays (20,000 r) and of thermal neutrons (6 hrs. at 4.65×10^8 N_{th}/cm.^{2}/sec.). These two irradiated lots gave about the same average decrease in seedling height. This serves to exemplify the different response of dormant barley seeds to these two types of radiations.

The treated material was permitted to continue growing in the greenhouse for 40 days following planting. At the end of that time differential killing of seedlings was apparent in the treatments receiving the three highest doses of x-rays. However, in the material irradiated with thermal neutrons, growth had ceased rather uniformly throughout the highest dosage (12 hrs.). At all other doses of thermal neutrons, growth was continuing and there was no evidence of any killing.

Chromatinic bridges and fragments in anaphase cells from root tips of irradiated dormant seeds were used to estimate the chromosomal damage resulting from the irradiations. Data from the various treatments used are presented in tables 1 and 2. The highest dosage of x-rays resulted in a bridge and fragment frequency which was about the same as that obtained with a 3-hr. thermal neutron treatment. The highest dosage of thermal neutrons resulted in a bridge frequency of 1.5 per cell, which is about twice as great as has been noted previously with a lethal x-ray dose of 32,000 r (unpublished results).

TABLE 1

THE EFFECT OF THERMAL NEUTRONS ON FREQUENCIES OF BRIDGES AND FRAGMENTS IN TREATED DORMANT SEEDS OF BARLEY (ALL SEEDS WERE SUBJECTED TO A FLUX OF 4.65×10^8 N_{th}/CM.^{2}/SEC. FOR THE TIMES DESIGNATED)

TREATMENT	CELLS OBSERVED, NO.	BRIDGES PER CELL, NO.	FRAGMENTS PER CELL, NO.	ABNORMAL CELLS,[a] %
Control	114	0	0	0
3 hrs.	102	0.48	1.4	46
6 hrs.	88	0.79	1.8	65
9 hrs.	93	1.23	3.1	81
12 hrs.	96	1.53	4.6	92

[a] Cells with one or more bridges or fragments were considered abnormal.

TABLE 2

THE EFFECT OF X-RADIATION ON THE FREQUENCIES OF CHROMOSOMAL BRIDGES AND FRAGMENTS IN TREATED DORMANT SEEDS OF BARLEY (ALL DOSES GIVEN AT 250 KVP. AND 30 MA.)

TREATMENT	CELLS OBSERVED, NO.	BRIDGES PER CELL, NO.	FRAGMENTS PER CELL, NO.	ABNORMAL CELLS,[a] %
Control	102	0	0	0
5,000 r	402	0.13	0.33	19.6
10,000 r	494	0.30	1.1	34.2
15,000 r	139	0.37	1.3	41.6
20,000 r	106	0.48	1.4	50.3

[a] Cells with one or more bridges or fragments were considered abnormal.

Discussion.—Heretofore, studies comparing the effects of x-rays and thermal neutrons have indicated that these two types of radiations produce comparable biological effects, although their physical properties are vastly different. To the writers' knowledge this is the first demonstration that these two radiations may produce biologically different reponses. This is most evident in the distribution of individual seedling heights following irradiation of seeds with x-rays and thermal neutrons (see Figs. 1–3).

These observations show that individual barley seeds subjected to identical doses of x-rays may be variously affected, whereas with identical doses of thermal neutrons they are injured much more uniformly.

When seeds are subjected to x-rays, events causing ionizations should be randomly distributed. With thermal neutrons this is not the case because some atomic nuclei have markedly higher cross sections for this type of radiation than do others. Thus, it seems possible that similar nuclear events have a greater chance of occurring equally in seeds subjected to thermal neutrons than in seeds subjected to x-rays. This may partially explain the seedling injury patterns reported here.

For years radio-biologists have been accustomed to obtaining differential survival of any class of organisms subjected to sufficient x-radiation to induce some killing. The reasons for this variation have been attributed to diverse factors including both genetical and physiological phenomena. With irradiated dormant seeds the writers had previously been of the opinion that the chance accumulation of chromosomally aberrant cells was a prime factor in differential killing. This is now considered unlikely, because with thermal neutrons it has been possible to obtain much higher aberration frequencies than was possible with x-rays before any killing took place.

For equal numbers of chromosomal aberrations thermal neutrons caused less seedling injury than x-rays. If this observation is interpreted on a cellular basis, it indicates that a considerable amount of x-ray-induced injury must be due to ionizations in extra-chromosomal elements of the cell. This is not intended to imply that thermal neutrons do not have some effect on such extra-chromosomal elements. However, it indicates that, in comparison with x-rays, proportionately more events occur in the chromosomal than in the extra-chromosomal elements of the cell.

If one assumes that ionization in either the chromosomal or extra-chromosomal constituents of the cell may be manifested in reduced growth, and that the two may be additive, the data for the x-ray-treated seeds are more readily understandable. Due to chance, either chromosomal or extra-chromosomal constituents could be affected in varying degrees and this could account for the divergent seedling heights obtained from seeds given the same dosage of x-rays.

Summary.—Dormant seeds of Himalaya barley were subjected to various doses of either x-rays or thermal neutrons. Seedlings from seeds treated with x-rays had a wide range in height distributions at 14 days, whereas those from thermal neutron irradiation had a narrow range.

Differential killing was a characteristic of the material subjected to the three highest doses of x-radiation. Such a lethality pattern did not occur in the thermal neutron treated material, i.e., if any seedlings survived a treatment, essentially all of them did.

For similar chromosomal aberration frequencies there was less seedling

injury in seeds subjected to thermal neutrons than in seeds treated with x-rays. Apparently x-rays have proportionately more effect on the extra-chromosomal elements of the cell than do thermal neutrons.

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THE EFFECTS OF CARBON DIOXIDE ON THE FREQUENCY OF X-RAY INDUCED CHROMOSOME ABERRATIONS IN TRADESCANTIA

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The role of oxygen as a factor in radiosensitivity of various cells and tissues has been the subject of considerable study (Sparrow and Rubin,[1] Giles and Riley,[2] Conger and Fairchild,[3] King, Schneiderman and Sax[4]). Little is known concerning the influence of another equally important molecule—carbon dioxide. Although carbon dioxide has for a long time been associated only with photosynthesis and as an end-product of respiratory metabolism, it has become increasingly clear since the classical experiments of Wood and Werkman[5] that carbon dioxide is vitally concerned in the biochemical activity of cells, particularly in synthetic reactions. The experiments to be reported show that carbon dioxide plays a significant role in radiosensitivity and thus reemphasizes the biochemical importance of this gas.

Materials and Methods.—*Tradescantia paludosa* Anderson and Woodson was used in all experiments. Cytological observations were made of chromosomal aberration types 5 days after irradiation. The principal