THE RELATION OF ATMOSPHERE TO BIOLOGICAL EFFECTS OF X-RAYS[1]

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FOR most of the past half century since the discovery of radium and X-rays, the effects of radiations from these sources upon living cells have been studied intensively. Although much information has been accumulated on the macroscopic and cytological changes produced, little is known of the mechanics of these changes or of the relation of environmental conditions to the effects of short-wave radiations. The present study is a contribution to the meager observations already recorded on the relation of atmosphere to the biological effects of X-radiation.

An effort was also made to determine the presence or absence of correlation (or cause and effect relationship) between injury symptoms, genetic effects, and chromosomal aberrations resulting from X-raying seeds (grains) of barley.

These experiments continue investigations begun by SMITH (1946 and unpublished).

LITERATURE REVIEW

Literature on the effects of X-rays on living cells has been summarized by DUGGAR (1936), GOODSPEED and UBER (1939), and LEA (1947), among others. Heavy doses of X-rays reduce viability, retard germination (both rate and percentage), stunt growth, and increase mutations and sterility. According to FRÖIER and GUSTAFSSON (1941) and GUSTAFSSON (1947), X-rays cause two types of cytological changes depending upon whether the nuclei are in interphase or actual division when irradiated. The primary effects, arising when irradiation is applied during prophase, are irregular fragmentation and an agglutination of the chromosomes resulting from "stickiness" of the matrix. As a consequence of this agglutination, the movements of the chromosomes are irregular and may lead to an atypical orientation of the spindle, degeneration of daughter nuclei, pseudo-amitoses, extension in time of contraction of chromosomes before metaphase, and other related aberrations. The secondary effect produced by irradiation of interphase nuclei, stems from aberrations resulting from fragmentation with or without subsequent rejoining of the fractured chromosome ends. It gives rise to translocations, inversions, deletions, bridges, fragments, and related irregularities. It is this second effect which causes genetic changes, but it has yet to be determined whether it is the first, the

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second, or some other effect which is primarily responsible for the physiological changes (injury symptoms).

Literature on the influence of atmosphere on X-ray effects is limited, but apparently there is less injury in the absence than in the presence of air. MOTTRAM, in 1935, reported that there was less inhibition in the growth of roots of *Vicia faba* if they were X-rayed in anaerobic rather than in aerobic conditions. The work of SMITH (1946 and unpublished) and THODAY and REED (1947) confirmed these results. SMITH mentioned preliminary data which indicated that there was less injury to dormant seeds of barley and einkorn when they were X-rayed in a vacuum or in an atmosphere of nitrogen than when they were X-rayed in air. This type of knowledge may also be of interest in X-ray therapy, as suggested by THODAY and REED (1947) who demonstrated that dividing cells were less injured if irradiated in the absence of air. Their results were considered to confirm observations of CRABTREE and CRAMER (1934) who had previously found "a similar effect of anaerobiosis on radiosensitivity of tumour cells." (Quoted from THODAY and REED 1947.)

One concept held by many authors is that damage from X-radiation is in general proportional to the dosage. TIMOFÉEFF-RESSOVSKY (1937, 1939) and others have felt that the number of genic changes induced by X-rays are directly proportional to the dosage and are independent of the wave length or time factor. BAUER (1939) stated that the number of rearranged or reunited contact points was proportional to the square of the X-ray dose. CATCHESIDE (1938) X-rayed *Drosophila melanogaster* males and noted that the number of structural changes induced in chromosomes showed a direct linear proportionality to the dosage between 1,000 r and 4,000 r. Later MULLER (1940), among others, concluded that the frequency of chromosomal aberrations increased somewhat more than the first power and less than the square of the dosage. SAX (1938) presented data showing that the frequency of chromosomal aberrations increased geometrically with X-ray dosage. These statements are indicative of the lack of agreement among authors as to the nature and relation of genic and structural effects induced by irradiation. Other works have been summarized in the discussion in this paper.

A second concept of concern to this report is the belief held by many workers that X-ray injury and lethality are due to genetic causes. FRÖIER and GUSTAFSSON (1941) stated that since chromosomes are the only parts of the cell with continued independent existence, any chromosomal disturbance would influence cell division and hence cell reproduction and related phenomena such as growth. They stated further that profound nuclear and chromosomal alterations are irreversible, whereas induced cytoplasmic changes can probably be reversed. Therefore, they concluded that seed mortality following irradiation is "probably an expression of chromosomal and genic changes." LEA (1947, pp. 341–344) was more cautious. He reasoned that since X-radiation kills bacteria and large viruses in the same proportions and under the same conditions that produce gene mutations in *Drosophila*, lethal action in bacteria and viruses might be the result of lethal gene mutations. However, he only suggested that such could be the situation in higher organisms. There is no proof as yet for

such a hypothesis, but on the other hand, there is evidence that chromosome structural changes cause the death of rapidly dividing cancer cells in which degeneration sets in only at the next division following irradiation.

THODAY and REED (1947) cited GRAY (1942) as being one of several authors who have hypothesized that the lethal effect of ionizing radiations is a result of abnormalities resulting from the division of cells with aberrant chromosomes. If this were the case, a decrease in injury symptoms should be accompanied by a parallel decrease in the number of chromosomal aberrations. In subsequent experiments in which roots of *Vicia faba* were X-rayed in three different media (oxygen, air, and nitrogen), they found that roots irradiated in oxygen grew less, and those treated in nitrogen grew more, than those treated in air. Correspondingly, there were more chromosomal abnormalities (bridges and fragments) in those cells irradiated in pure oxygen than in those treated in nitrogen. Similar results were obtained when carbon dioxide, nitrous oxide, and hydrogen were substituted for nitrogen. This suggests that even though physiological injury is ordinarily correlated with chromosomal changes, there must be some physical factor present which influences the degree to which X-rays damage the cells. THODAY and REED'S experiments most strongly point to oxygen and MOTTRAM'S (1935) and SMITH'S (unpublished) findings further suggest this possibility.

Other workers have also indicated that there may be factors involved in injury resulting from irradiation, other than merely genetic changes—for example, a chemical, physiological, or physical effect. FRÖIER and GUSTAFSSON (1941), and KEMPTON and MAXWELL (1941), among others, mentioned that there is a delayed killing action following irradiation that would be somewhat difficult to account for on the basis of induced genic changes, which presumably are more or less instantaneous. However, some chromosomal aberrations might develop their full effects only after several cell generations. According to FRÖIER, GELIN, and GUSTAFSSON (1941), nuclei of *Avena sativa* will divide even when the chromosomes are so distorted that they have lost their characteristic rod-like shape. They suggested that the extra-chromosomal influence that initiates mitosis may be inhibited by extremely high doses of irradiation. FRÖIER and GUSTAFSSON (1941) noted that KAPLAN (1940) had reported that pollen-tube nuclei of *Antirrhinum* were inactivated by 100,000 r, whereas pollen tube growth was possible after a treatment of 300,000 r. They suggested that perhaps the effect of such heavy doses of irradiation is extra-nuclear and probably chemical. They refer to KAPLAN'S (1940) theories: that irradiation causes ionizations which may destroy compounds of low molecular weight and give rise to poisonous compounds, or, that the rays may destroy the "Steuerungszentren" which are large molecules responsible for catalyzing and regulating physiological processes.

Specifically, the physiological factor in X-ray injury may be the inactivation of auxin or growth substances, or possibly even enzymes. FRÖIER and GUSTAFSSON (1941) noted that wet pollen was less susceptible to X-ray injury than dry pollen, and suggested that this resistance resulted from the ability of hydrated spores to replace auxin destroyed or inactivated by irradiation. SKOOG (1935),

in an outstanding work on the effect of X-radiation on auxins, noted that auxin was inactivated or destroyed by X-radiation and that the rate of inactivation was directly influenced by the presence of oxygen and the amount of auxin present. He then suggested that destruction of auxin is a major cause in the immediate inhibition of growth by X-radiation. GOODSPEED and UBER (1939) cited CHOLODNY (1935) as also suggesting that dwarfing following X-radiation is probably caused by inactivation of auxin. POPP and MCILVAINE (1937) showed, in a study using various long energy wavelengths between 250 mμ and 450 mμ that the reduction in growth substances was correlated with a decrease in the wave length of the incident radiation. In his summary of the biological effects of radiations on living cells, LEA (1947 p. 37) brought out the possibility of the inactivation of vital enzymes by irradiation, pointing out that enzymes in the dry state are inactivated by X-radiation.

Thus, whether there is a necessary (cause and effect) relation between genetic aberrations and physiological injury resulting from X-radiation is a matter of debate. Usually physiological reaction and frequencies of genetic changes are correlated, but critical experiments capable of distinguishing whether the correlation is chance or necessary are not common. SAX (1942) concluded that while chromosomal alterations may be the chief cause of injury, the physiological response of tissues to X-rays may be important too. GELIN (1941), from his work with dormant and germinating seeds of barley, stated that "after X-raying with the same dosage, the reaction of the chromosomes is directly influenced by the physiological condition of the seeds."

MATERIAL AND METHODS

In all experiments reported herein, seeds of Himalaya barley were used. The seeds were soaked in water about eight hours, after which they continued germination on moist blotters in Petri dishes for about ten hours. The water and Petri dishes were kept at room temperature. Germinating seeds were used for two reasons: 1) at the time the experiments were begun the X-ray apparatus available was not satisfactory for giving dosages large enough for efficient use of dormant seeds; and 2) because a given dose of X-rays produces a much higher frequency of changes in germinating than in dormant seeds. SMITH (1946) cited several authors who have demonstrated that the effect from irradiation is greatly increased with increased moisture content of seeds. FRÖIER and GUSTAFSSON (1941) noted that with desiccation there is a stabilization of nuclei, whereas, conversely, hydration increases nuclear lability to radiation (GELIN 1941). GELIN reported that an increase of 5 percent in water content of seeds (from 10 percent to 15 percent) increased chromosomal aberrations twofold. He suggested that possibly water weakens the bonds between atoms by penetrating, or being incorporated into, the chromatinic protein molecules.

Comparisons between the effects of a given dose of X-rays on seeds in air and in a vacuum were made as follows: the same number of seeds were placed in two similar pyrex test-tubes (taken from the same commercial carton). The tubes were tested and found to absorb approximately 60 percent of the irradiation so that with treatments of 3,000 r, for example, the seeds actually received

about 1,200 r. Hereafter, however, the doses reported are those applied at the surface of the seeds. Seeds that were to be irradiated in air were treated simultaneously with those that were sealed in a vacuum in the second tube. The vacuum was created by a suction pump designed to reduce the pressure to 0.0001 mm Hg. Although the degree of vacuum was never measured, results depending on this factor were consistent. While in the vacuum (insured by a greased stopcock) the seeds were carried $\frac{1}{8}$ mile to the X-ray machine. Every experiment included a control sample of seeds.

In several instances seeds in a third test-tube were X-rayed simultaneously for one or the other of the following additional treatments: 1) seeds were X-rayed in air after being in a vacuum for a period of time equal to the time the lot of seeds were in the vacuum during irradiation; or 2) seeds were X-rayed and then exposed to a vacuum for a corresponding period of time.

An X-ray machine operated at about 230 KVP and 3 ma was used for all the tests. The seeds were $7\frac{1}{2}$ inches from the target of the tube and received 50 r to 65 r per minute. Varying dosages were given ranging from 400 r to 5,200 r.

After being irradiated, the seeds were planted immediately. Germination was measured by the number of seedlings that emerged, and growth by the estimated average height. These data were obtained by growing the seeds in loam (one flat per test run in the greenhouse). The height was estimated by placing a ruler beside the clumps of seedlings. In one case additional data on germination were obtained when the seeds were planted in the field for a mutation study. Heat from a 200-Watt light bulb was applied in a few of the earlier tests to hasten growth processes.

To determine the seedling mutation rate, plants from treated seeds were grown to maturity. In the fall, seeds from individual spikes of these plants were grown in the greenhouse and the mutation rate was determined following the method of STADLER (1930). The mutation rate was based on the number of observable mutants appearing among 3- to 4-inch seedlings during a growth period of from ten days to two weeks. In some cases more than one kind of mutant was present among the progeny of a single spike. In such an event each different type of mutant was counted as an independent mutation.

To obtain cytological data, seeds were germinated on moist blotters in Petri dishes. When the roots were $\frac{1}{8}$ inch or less in length, they were fixed in Carnoy's fluid, formula A, and stained with acetocarmine following the technic described by SMITH (1947). Such a smear technic has especial advantage for this kind of study because it is rapid and because individual cells are isolated more or less in a single plane. It also avoids a pitfall of sectioning technics—namely, artificial production of fragments.

Chromatinic bridges at late anaphase or early telophase were used as the index of frequency of chromosomal changes. Bridges were used as criteria because they can be seen with considerably greater certainty than fragments. However, fragments were tabulated, because, though not so accurate, their frequencies did give indications that supported the data obtained on the frequencies of bridges.

EXPERIMENTAL RESULTS

A. The Effect of Irradiation in Air and in a Vacuum on Germination and Growth

In the first test, samples of 50 seeds each were given the following treatments: 1) one lot was left as a control, 2) a second was subjected to a vacuum for one hour, 3) a third was X-rayed while surrounded by air, 4) a fourth was X-rayed while in a vacuum, and 5) a fifth was subjected to a vacuum for one hour and then X-rayed in air. In order to determine the relation between X-ray dosage and atmosphere, four different dosages of X-radiation were ap-

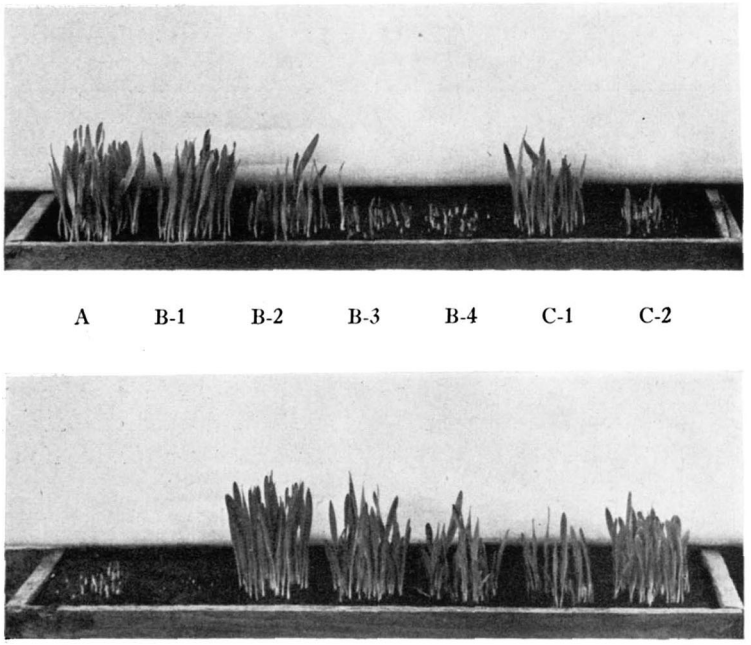

FIGURE 1.—A comparison of the effects of X-raying germinating barley seeds in air and in a vacuum as indicated by 14-day-old seedlings. A, Control. B, X-rayed in air: 1—400 r; 2—800 r; 3—1 200 r; 4—1,600 r. C, X-rayed in air after evacuation: 1—400 r; 2—800 r; 3—1,200 r; 4—1,600 r. D, X-rayed in a vacuum: 1—400 r; 2—800 r; 3—1,200 r; 4—1,600 r. E, Evacuated only.

plied to additional series: in one series the irradiation was 400 r, in the second 800 r, in the third 1,200 r, and in the fourth 1,600 r. The data from this experiment are presented in table 1. Figure 1 shows part of the plants from this same experiment. From this and other experiments, 1,200 r was chosen as a suitable dosage to show the contrast between irradiation in air and in a vacuum, and was used in most of the later tests.

Table 2 presents a summary of two succeeding tests. In addition to the treatments mentioned in test 1, a sixth sample of seeds was X-rayed in air and

TABLE 1

A comparison of the effects of X-raying germinating barley seeds in air and in a vacuum as measured by germination and growth.

X-RAY DOSAGE	GERMINATION (PERCENT)[1]					HEIGHT OF SEEDLINGS[2]				
	0	400 r	800 r	1,200 r	1,600 r	0	400 r	800 r	1,200 r	1,600 r
Treatment[3]										
Control	100					100				
Evacuated only	96					96				
X-rayed in air		100	100	92	94		96	31	11	4
X-rayed in a vacuum		96	100	96	88		100	88	81	62
X-rayed in air after evacuation		100	85	67	29		57	19	8	2

[1] Actual percentage of seeds germinated.

[2] After 14 days (percent of control).

[3] Applied to 50 seeds in each sample (a total of 700 seeds were involved in the experiment).

then subjected to a vacuum until the seeds had been without air for as long as those that were X-rayed in a vacuum had been.

The results indicated that in general those seeds which were X-rayed while in a vacuum had a higher germination percentage and in every case a better growth rate than those X-rayed in air. This trend was consistent in each of the 12 trials run to show effect on germination and growth, the data from three of which are presented in tables 1 and 2. In table 2 it can be noted that the height of seedlings from seeds X-rayed in air either before or after a period of time

TABLE 2

A further comparison of the effects of X-raying germinating barley seed in air and in a vacuum as measured by germination and growth. (Data from two tests.)

AGE OF SEEDLINGS	GERMINATION (PERCENT)[1]		HEIGHT OF SEEDLINGS[2]	
	Test A 11 days	Test B 12 days	Test A 11 days	Test B 12 days
Number of seeds in sample	50	25	50	25
Treatment				
Control	100	80	100	100
Evacuated only	98	—	111	—
X-rayed[3] in air	24	4	37	11
X-rayed in a vacuum	98	80	56	79
X-rayed in air after evacuation[4]	32	4	59	63
X-rayed in air before evacuation[4]	20	4	59	37

[1] Actual percentage of seedlings germinated.

[2] Percentage of control.

[3] 1,200 r units.

[4] Vacuum applied 35 minutes in test A and 60 minutes in test B.

in a vacuum was not reduced so much as those X-rayed in air without evacuation. However, the survival of seeds given the three treatments were comparable. In table 1 the relation between treatments and seedling heights are more nearly as expected. From table 1 and figure 2 it is evident that an inhibition of growth preceded a decrease in germination, that is, there was a much greater gap between growth of seeds given 400 r of X-rays in air and those given 1,600 r than there was in the corresponding germination percentages. Thus, germination dropped only 6 percent as the irradiation increased from 400 r to 1,600 r, while the growth was reduced by 96 percent.

It might easily be possible that a vacuum could modify X-ray effects by any one of several indirect influences, such as injury to cells from lack of oxygen; removal of water from the seeds as it vaporized in the reduced pressure during evacuation, making them less susceptible to X-ray injury; or the change in gaseous pressure. To test the effect of evacuation itself on germination and chromosome injury, a test-tube containing 20 seeds was attached to a vacuum pump which was left running for three hours. The seeds were then germinated in Petri dishes, and root tips were taken for cytological observations. Both the treated seeds and a control showed 100 percent germination. No chromosomal aberrations were observed among 110 cells examined from the vacuum-treated seeds. Additional proof that evacuation alone did not cause observable deleterious effects is evident in tables 1 and 2 where the seeds subjected to a

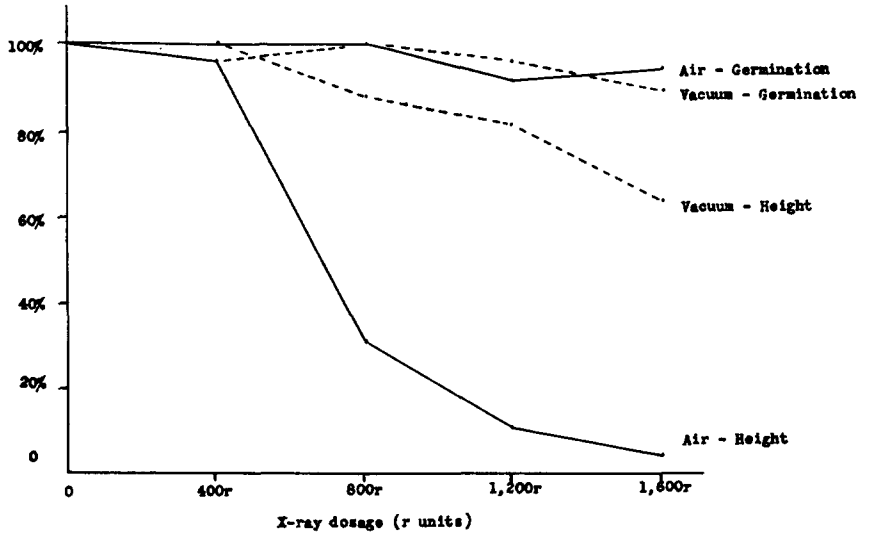

FIGURE 2.—A graph comparing the effects of X-raying germinating barley seeds in air and in a vacuum as measured by germination and growth (height).

vacuum grew about as well as the controls. Keeping seeds in a vacuum for a time might reduce the amount of moisture in them, and as a result make them less susceptible to X-ray injury than seeds that had been soaked an equivalent amount of time and X-rayed in air. There are two reasons for doubting that the vacuum removed enough water to make any significant difference in the

results obtained: 1) in all of these experiments the test-tube containing the seeds was attached to the vacuum pump no more than five minutes while it was in operation, and 2) unpublished data of SMITH showed that keeping dormant seeds of wheat and barley in a vacuum for three hours removed no more than 0.25 percent by weight of moisture. Finally, data from this same paper indicated that pressure alone within the tubes was not the controlling influence on the X-ray effects. This conclusion was suggested by the fact that replacing the air with nitrogen resulted in reduced injury symptoms comparable with those obtained from X-raying seeds in a vacuum. Moreover, a limited amount of data suggested that replacing air with pure oxygen at increased pressures did not increase the injury over that resulting from irradiation in air at normal pressures.

An effort was made to determine the lethal dosages for germinating seeds irradiated in air and in a vacuum, and to determine the dosages given to seeds treated in air that would be equivalent to dosages given seeds treated in a vacuum. The tests are incomplete, but limited observations indicated that the lethal dose for seeds treated in air was approximately 4,000 r, whereas seeds treated in a vacuum survived 5,200 r.

B. The Effect of Irradiation in Air and in a Vacuum on Mutation Rate

In order to compare the mutation rates of seeds irradiated in air and in a vacuum, several samples of 100 or 150 seeds each were irradiated in air simultaneously with numerically equivalent samples irradiated in a vacuum. The treatment was applied in April, 1947, and the seeds were then grown in the field. Whole plants were harvested by pulling in late July. Table 3 presents the results on seedling mutation rates determined by growing head progenies of these plants in the greenhouse in the winter of 1947–48.

The mutation rate obtained from irradiating seeds in air was 3.4 percent as compared with 2.1 percent for those irradiated in a vacuum. The standard

TABLE 3

A comparison of the effects of X-raying (1,200 r) germinating barley seeds in air and in a vacuum as measured by mutation rate and related data.

	TREATMENT		
	CONTROL	X-RAYED IN AIR	X-RAYED IN A VACUUM
Number of seeds treated	500	2,000	2,000
Seeds survived (Percent)	94	60	79
Weight at harvest (Total)	22,400g	36,625g	54,975g
Weight per plant (Average)	47.8g	30.3g	34.6g
Number of head progenies tested		3,425	3,309
Number of progeny per head (Average)		30.7	31.0
Number of mutants (Total)		118	70
Heads with mutants (Percent)		3.4	2.1

Standard error of difference (3.4−2.1) = 1.3 ± 0.4%

error of the difference was 0.4 percent, indicating that the mutation rate in seeds X-rayed in a vacuum was probably significantly lower than the rate in seeds given a simultaneous irradiation while in air. It is apparent that for some reason the vacuum gave a measure of protection to the seeds against mutation, stunting, and reduction in germination.

The types of mutants and the proportions of these types resulting from irradiation in air and in a vacuum are of interest (table 4). Mutations involving self-color changes ranged from white through yellow to yellow-green. Variegated colors included transverse zoning, horizontal striping, and virescence. Though less common in occurrence, other mutant types such as shriveled (necrotic) bands, shriveled tips, or variations of these, appeared. In general, there was a slightly greater percentage of the more extreme types of mutants,

TABLE 4

A comparison of the proportions and types of seedling mutants obtained from X-raying (1,200 r) germinating barley seeds in air and in a vacuum.

FROM SEEDS IRRADIATED IN	MUTANTS (NUMBER)		PERCENT OF TOTAL		FREQUENCY[1] (PERCENT)	
	AIR	VACUUM	AIR	VACUUM	AIR	VACUUM
Mutants (types)						
White	71	30	60	43	15	12
Yellow	11	4	9	6	15	13
Yellow-green	13	15	11	21	21	8
Virescent	10	8	9	11	15	17
Miscellaneous.						
Striped	3	8	3	11	5	7
Banded shrivel	1	4	1	6	25	16
All others	9	1	8	1		
Totals	118	70				

[1] Average percent of mutant seedlings per head progeny.

such as white or yellow seedlings among the progeny from parents X-rayed in air, whereas less extreme mutant types such as yellow-green seedlings seemed to form a greater proportion of the types obtained from seeds exposed while in a vacuum. For example, white seedlings were found to compose 60 percent of the mutants from seeds X-rayed in air and only 43 percent from seeds treated in a vacuum, while, conversely, yellow-green mutants composed 11 percent of the total from irradiation in air and 21 percent from irradiation in a vacuum. Also, there was a slight indication that more seeds per head gave rise to mutant seedlings in those spikes from seeds irradiated in air than from those X-rayed in a vacuum. However, it is apparent from table 4 that there were not enough mutants in most comparisons to give more than suggestive results.

C. The Effect of Irradiation in Air and in a Vacuum on Chromosomal Aberrations

In tables 5 and 6 are summarized the results obtained from cytological examinations of root-tip cells. It is evident that the frequencies of bridges found

TABLE 5

A comparison of the frequencies of chromatinic bridges in cells of root tips grown from germinating barley seeds X-rayed (1,200 r) in air and in a vacuum.

TESTS MADE	AGE OF ROOT TIPS (AFTER RAYING)	TOTAL NUMBER OF CELLS EXAMINED		NUMBER OF BRIDGES PER CELL		STANDARD ERROR OF DIFFERENCE	RATIO OF BRIDGES PER CELL
		AIR	VACUUM	AIR	VACUUM		AIR:VACUUM
Test No. 1	24 hours	99	100	.24	.08	.051	3:1
Test No. 2	30 hours	100	83	.44	.08	.051	5:1
Test No. 3	29 hours	37	27	.19	.07	.077	2:1
Test No. 4	26 hours	16	47	.37	.04	.031	7:1
Totals		252	257				
Weighted average							4.7:1

in cells from root tips of seeds X-rayed in air consistently exceeded the frequencies found in cells from root tips of seeds given the X-ray treatment while in a vacuum. Also, it may be pointed out that there was a greater difference between the frequencies of bridges obtained from treatments in air and in a vacuum than was obtained between the mutation rates. In some cases (table 6), the frequency of bridges from root tips of seeds X-rayed in air was seven times the frequency of bridges found in cells X-rayed in a vacuum, whereas the mutation rate for those seeds treated in air was only 1.6 times the frequency obtained from seeds irradiated while in a vacuum.

TABLE 6

A further comparison of the frequencies of chromatinic bridges in cells of root tips grown from germinating barley seeds X-rayed (1,200 r) in air and in vacuum.
(Summary of four tests distributed over a period of three months.)

TREATMENT	TOTAL NUMBER OF CELLS EXAMINED	FREQUENCIES OF CELLS WITH INDICATED NUMBERS OF BRIDGES					BRIDGES PER CELL (AVERAGE)	STANDARD ERROR OF DIFFERENCE
		0	1	2	3	4		
Control	71	71					0	
Evacuated only	147	147					0	
X-rayed in air	252	202	24	22	3	1	0.30	0.03
X-rayed in a vacuum	257	242	12	1	2		0.08	

DISCUSSION

The relation of atmosphere to the biological effects of X-rays is of interest because it may throw some light on the mechanism by which short-wave radiations produce biological effects. It was hoped that these experiments would help distinguish between the belief of GRAY (1942), LEA (1947 p. 342) and other workers, that injury and death resulting from irradiation are due primarily to genetic aberrations, and the possibility, as suggested by KEMPTON

and MAXWELL (1941), among others, that injury and death may be caused by extra-chromosomal changes. The present results and those of MOTTRAM (1935), SMITH (1946), and THODAY and REED (1947) suggest that X-ray effects (perhaps both chromosomal and extra-chromosomal), may be modified by external conditions, but they do not distinguish between the importance of genetic and physiological factors in biological effects of X-rays.

The results herein reported show clearly that, if the seeds are not in air when X-rayed, they are damaged less (physiologically, genetically, and cytologically) than if they had been in the presence of air when X-rayed. Apparently, a vacuum either exerts a protective influence by keeping the rays from reaching the seeds or there is in the air some component that, in conjunction with irradiation, or activated by it, augments the damage. The first possibility is highly improbable, and the results obtained by MOTTRAM (1935), SMITH (1946 and unpublished), and THODAY and REED (1947) seem to point to the same conclusion: that something in the air, possibly oxygen, increases the injury from irradiation. One hint as to the possible mechanism by which atmosphere may influence X-radiations has been advanced by SKOOG (1935). He showed that auxins are destroyed when X-rayed in the presence of air and presumably this results from an interaction between the X-rays, some component of the air, and the growth hormones—perhaps an oxidation reaction. It is also possible that enzyme inactivation by X-rays (LEA 1947) may be a contributing factor.

There is evidence (STEINITZ 1943) that the absence of oxygen for four days has deleterious effects on barley seedlings. But the data herein show just the opposite trend, so this effect can probably be ruled out in the tests reported here for the short time that the seeds were without oxygen. In spite of the fact that STEBBINS and STEINITZ (1939) had shown that the absence of oxygen in the surrounding atmosphere caused chromosomal aberrations in barley seedlings, THODAY and REED (1947) substituted nitrogen for oxygen or air while roots were being irradiated and obtained reduced frequencies of chromosomal aberrations resulting from the irradiation. Presumably the deleterious effect from keeping seedlings in a nitrogen atmosphere could be neglected since the seeds were not exposed for long periods of time.

Many factors of various natures have been reported to affect the biological effects induced by a given dose of X-radiation. Perhaps the factor that has received most attention has been temperature, about which reports are at variance. KEMPTON and MAXWELL (1941) reported that the maximum sensitivity of air-dried maize seeds to X-rays existed between 0°C and room temperature, with a reduction in sensitivity correlated with either a decrease or increase of temperature outside of this range. (The temperature was maintained during irradiation.) Even when the seeds were held at the temperature of liquid air during treatment the sensitivity was decreased. However, MAXWELL, KEMPTON, and MOSLEY (1942) reported that when cold was applied after irradiation, or heat applied before, the sensitivity of seeds of maize to X-ray injury was increased. Working with the lower side of the temperature scale, TASCHER (1929) and STADLER (1931) reported that extreme cold ob-

tained by packing seeds in solid carbon dioxide reduced sensitivity of dormant barley seeds to X-radiation. Although STADLER (1930) found that temperature had no effect on the production of mutations by X-rays, SMITH and CALDECOTT (1948) observed that the mutation rate of dormant seeds resulting from X-radiation was increased with pre- or post-treatments with heat. These same authors found that the frequency of chromosomal bridges was reduced by application of sub-lethal heat treatments to barley seeds before or after irradiation. These results support evidence of SAX and ENZMANN (1939) who noted a decrease in frequencies of structural changes with an increase in temperature during and after irradiation. The temperature was thought not to have any effect on the actual production of chromosomal breaks, but only on the recombination of broken chromosome ends. FABERGÉ (1940a) stated that high temperature with irradiation reduced the number of fragments. CATCHESIDE *et al.* (1946b) showed that though the number of chromatid breaks (a break in only one of the chromatids of a chromosome) was slightly correlated with an increase in temperature accompanying irradiation, the number of iso-chromatid breaks ("a fracture of a whole chromosome into two parts, both chromatids having been broken at apparently the same locus," CATCHESIDE *et al.* 1946a) was definitely affected by an increased temperature. SAX (1947) found that low temperatures during irradiation increased the frequency of chromosomal aberrations, whereas low temperatures before or after X-ray treatment had no effect.

A second factor that affects biological effects of X-radiation, and about which quite a lot of information has been accumulated, is moisture content. GELIN (1941), FRÖIER and GUSTAFSSON (1941), STADLER (1930), TASCHER (1929), and many others have noted that increasing the moisture content of seeds increases the sensitivity of those seeds to X-ray injury. It has been observed also by GELIN (1941) and others that increased moisture content is correlated with increased frequency of chromosomal aberrations from a given dose of X-rays. Similarly, STADLER (1930, 1931) and GUSTAFSSON (1947) observed that a higher mutation rate was obtained per r unit applied to germinating seeds as compared with dormant seeds.

It has been found that other wave lengths of energy modify the effects of X-rays. HOLLAENDER and SWANSON (1947) reported that a pretreatment of Aspergillus and Trichophyton spores with near-infrared rays increased the frequency of X-ray-induced mutations, whereas infrared alone produced no mutations. KAUFMANN and GAY (1947), summarizing the results from a number of studies by KAUFMANN and several co-workers, reported near-infrared radiation, applied before an X-ray treatment, increased the frequency of chromosomal rearrangements but had no significant effect on the production of recessive, sex-linked lethal mutations. On the other hand, a summary of the work of DEMEREC *et al.* (1942) in which male flies of *Drosophila melanogaster* were exposed to infrared or ultraviolet radiation between two X-ray treatments, showed that the yield of chromosomal structural changes was reduced even though infrared or ultraviolet had no effect when used alone. SWANSON (1944) found that, within limits, an ultraviolet treatment of Tradescantia

pollen tubes before or after X-radiation led to a reduction in the number of chromosomal breaks from a given X-ray dosage.

Additional factors which have been reported to modify X-ray effects may be mentioned briefly. In addition to the observations reported in this paper, MOTTRAM (1935), SMITH (1946), and THODAY and REED (1947) have shown that atmosphere has an influential role on the injury, genetic, and cytological effects of X-radiation. The degree of injury resulting from irradiation has been reported by FABERGÉ (1940a), TASCHER (1929), and SAX (1941b) to be influenced by fractionating the dose, though there have been other reports to the contrary. EBERHARDT (1939), cited by FRÖIER, GELIN, and GUSTAFSSON (1941) presented data indicating that the time factor, or number of r units per minute has some bearing on the degree of X-ray lethality and visible changes. SAX (1941a) and CATCHESIDE *et al.* (1946b) obtained results on the frequency of chromosomal aberrations similar to the findings of EBERHARDT. LEA (1947, p. 145) on the other hand, noted no evidence that the time factor influenced the X-ray-induced mutation rate. BRUMFIELD (1943) suggested that the frequency of chromosomal aberrations and the intensity of radiation may be directly correlated because of the fact that the number of illegitimate fusions depends on the time "breaks remain open." A second factor which facilitates non-homologous pairing or interchanges is the amount of movement of the chromosomes during irradiation, a fact which is supported by the evidence of SAX (1943) that centrifugation during X-irradiation of Tradescantia microspores resulted in a higher frequency of aberrations. FRÖIER, GELIN, and GUSTAFSSON (1941) interpreted EBERHARDT'S results as indicating a difference in effect between hard and soft rays, although STADLER (1930), FABERGÉ (1940b), and a number of other workers have found no such difference within rather wide limits of wave lengths.

Two chemicals which have been reported to modify the effects of X-rays are colchicine and ammonia. BRUMFIELD (1943) observed that onion root tips treated with colchicine before irradiation had one third as many aberrations as those irradiated without colchicine treatment. It was suggested that probably colchicine reduced the movement of the chromosomes in prophase so that, following breakage, restitution rather than rearrangement was favored. MARSHAK (1938a, b) reported that a dilute solution of ammonia applied to onion seedlings before irradiation decreased the number of aberrations appearing three hours later at anaphase.

The cytological composition of the cell influences the reaction to X-rays. Genes themselves may control X-ray susceptibility. SMITH (1942 and unpublished) found evidence of a gene for X-ray sensitivity in *Triticum monococcum*. There is an increase in chromosomal aberrations (bridges, translocations, and irregular cell divisions) associated with an increase in number of genoms (FRÖIER, GELIN, and GUSTAFSSON, 1941; GUSTAFSSON 1947; SMITH 1943 and 1946), though these aberrations prove less likely to be lethal when there are more chromosomes present.

There is some evidence of differences among chromosomes, and among stages in the life cycle of the same chromosome or organism, in their reactions

to X-rays. BAUER, DEMEREC, and KAUFMANN (1938) presented data indicating that a given dose of X-radiation applied to male flies caused fewer breaks in the Y than in the X chromosome of Drosophila, in a ratio approximating 80:100. There is variation in X-ray sensitivity with respect to the stage of mitosis as shown in the frequency of chromosomal aberrations (SAX 1938; SAX and ENZMANN 1939; BRUMFIELD 1943). Increased sensitivity is correlated with the phase of greatest chromosome activity. Consequently, SAX (1938) found meiotic prophase to be ten times more susceptible to X-ray-induced aberrations than the meiotic resting stage, and mitotic prophase to be two times more susceptible than its resting stage. In addition, sensitivity varies with the life cycle (GOODSPEED and UBER 1939) and with the age of the tissue (GUSTAFSSON 1947 and SAX 1942) which to some extent may be associated with the frequency of mitotic activity.

Delaying germination of irradiated onion seeds increased the frequency of aberrations (due possibly to the same physiological effect that age has), but had no effect upon the germination of irradiated onion bulbs (SAX 1941b).

There are still other factors or conditions that influence the biological reactions to X-radiation. For example, dormant onion bulbs are approximately ten times more susceptible to the same dose of X-rays than are dry onion seeds (SAX 1941b). GUSTAFSSON (1947) reported large seeds of barley and wheat capable of tolerating a heavier dose of irradiation than smaller seeds were able to. FRÖIER and GUSTAFSSON (1941) and FRÖIER, GELIN, and GUSTAFSSON (1941) reported that the hulls of "hulled" types of Avena and Hordeum gave seeds some protection against injury from X-radiation. Among seeds of different plants there is wide variance in sensitivity to X-rays (GUSTAFSSON 1947 and TASCHER 1929) and according to FABERGÉ (1940b) "Tradescantia chromosomes behave differently when X-rayed than do those of Drosophila."

Chromatinic bridges at mitotic anaphase presumably result from the fact that a chromatid has two kinetochores instead of one. Irradiation may produce changes that result in chromatids with two kinetochores in at least three ways: 1) two homologous or non-homologous chromosomes or chromatids may break forming four fragments; the broken ends of the two fragments containing kinetochores may join; 2) both chromatids of a chromosome already split into two strands may break with subsequent joining of the broken ends of the two fragments containing kinetochores; or 3) a single strand may break and subsequently split into two chromonemata whose broken ends may join forming a fragment with two kinetochores. In any case, the result is a chromosome (chromatid) with two kinetochores and one, or two akinetic fragments. A bridge is formed if the two kinetochores become destined (presumably by chance if they are not close together) for opposite poles.

In these studies there has been some correlation between injury symptoms, genetic effects, and chromosomal aberrations resulting from X-raying seeds of barley. The correlation between frequencies of genetic effects, as measured by seedling mutation rates, and chromosomal aberrations, as measured by chromatinic bridges was not close. This was pointed out above where it was noted that the mutation rate from X-raying seeds in air was 1.6 times the rate ob-

tained by irradiating seeds while in a vacuum; but the frequency of chromatinic bridges in root-tip cells from seeds X-rayed in air averaged 4.7 times the frequency of those similarly treated in a vacuum. There are two plausible explanations for the contrast between the result obtained on bridge and mutation frequencies: 1) It is probable that cells with aberrant chromosomes either fail to survive or produce smaller proportions of the plant than do cells with normal chromosomes changed only by a "point mutation." Since chromosomal aberrations that lead to bridge formation at mitotic anaphases tend to be eliminated in the ontogeny of plants, and since chromosomal aberrations increase with dosage of X-rays at a different rate than does mutation frequency, a doubling of the mutation rate would not necessarily be expected to accompany a doubling of the frequency of chromatinic bridges in root-tip cells. 2) It might be that the chromosomal aberrations and mutations are not caused by the same mechanism. The evidence on this point is not conclusive.

SUMMARY

1. Barley seeds X-rayed while in a vacuum consistently showed better germination and growth and a lower frequency of chromosomal aberrations than seeds X-rayed in air. In one test the seedling mutation rate from tests of plants grown from germinating seeds X-rayed in a vacuum was 2.1 ± 0.32 percent as compared with 3.4 ± 0.35 percent for seeds X-rayed in air. This difference was slightly more than three times the standard error.

2. Root-tip cells from seeds irradiated in air averaged 4.7 times as many chromatinic bridges as root-tip cells from seeds irradiated in a vacuum.

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