

MUTANTS PRODUCED BY X-IRRADIATION OF SPORES OF  
CHAETOMIUM GLOBOSUM AND A COMPARISON WITH THOSE  
PRODUCED BY ULTRAVIOLET IRRADIATION\*

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INTRODUCTION

Mutations have been produced by irradiating spores of *Chaetomium globosum* with soft x-rays from a Shearer tube operating on 12 to 15 kv. and with harder x-rays from a dental tube operating on 62 kv. In this paper the mutations produced by x-rays are classified and a general comparison made with those produced by ultraviolet irradiation (previously described in papers by McAulay, Plomley, and Ford, 1945; Ford, 1946; Ford, 1947; McAulay and Ford, 1947; and Ford, 1948).

In the later papers on ultraviolet experiments the mutants have been classified into two broad groups. The same division is made in this paper.

(a) *Lethal Mutants*.—These are mutants in which the growth of the colony ceases before it has become visible (Ford, 1948). Upon microscopic examination these colonies are found to possess marked characteristics easily recognizable. They are produced by ultraviolet irradiation, visible light, and x-rays; they also occur in the progeny of certain macroscopic mutants. They are produced in up to 100 per cent in some irradiation experiments. Less than 1 per cent occur in the controls.

(b) *Macroscopic Mutants*.—These are visible mutants in which the growth rate and form vary from the normal colony. Up to 62 per cent occur with short ultraviolet irradiation; in x-ray experiments 15 per cent occur; and with long ultraviolet and visible wave lengths up to 19 per cent are produced. With these also, less than 1 per cent occur in controls.

Detailed experiments on the relation between dose in roentgens and the percentage lethal mutants have been made with x-rays from the dental tube. It has been found that the production of the mutants is consistent with five quantum hits. It would appear from the form of the curve discussed later that in x-ray experiments more quantum hits are required than the two quantum hits consistent with the curves obtained for ultraviolet mutant production (McAulay, Ford, and Dobie). A difference in position of the sensitive

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spot which absorbs the radiation in different spores may result in too low a figure for the number of quantum hits (2) obtained with mutant production in ultraviolet experiments.

In the case of the macroscopic mutants produced by x-rays, the most striking feature is the scarcity of the mutant *K* which is produced selectively and in large numbers by short ultraviolet wave lengths (McAulay, Plomley, and Ford, 1945; Ford, 1946). In this respect the results obtained with x-rays are similar to those obtained with long ultraviolet wave lengths, which is a striking result suggesting that the production of the *K* is restricted to a limited region of the electromagnetic spectrum. An analysis of the pigmented mutants suggests that more are produced by x-ray irradiation of the fungal spores of *Chaetomium globosum* than are produced by ultraviolet irradiation.

#### EXPERIMENTAL

Spores of the fungus *Chaetomium globosum*, Fld, were used. As stated in earlier papers from this laboratory (1945, 1946, 1947), Fld is a stable mutant which was produced in an early ultraviolet irradiation experiment. In these papers the ultraviolet apparatus and experimental procedure were described.

In the x-ray experiments the spores were spread with a brush on a circular area of celluloid half an inch in diameter. Approximately 4,000 spores were spread for both experimental and control samples. After irradiation the colonies were obtained by single spore and dilution plate techniques which have been described in detail by McAulay, Plomley, and Ford (1945). Lethal mutants were all of single spore origin. Single spores were examined daily for germination, until it was clear that either the colony became visible or could be classified with certainty as a lethal mutant. In some cases the examination was continued up to 5 or 6 days. Visible colonies were plated on 2 per cent malt agar, each colony in a separate Petri dish, and incubated at 28°C. After about 3 weeks, an analysis was made of the matured colonies for macroscopic mutants.

In experiments on the dose-per cent mutant relation, spore samples were taken from the irradiated and control spreads, 100 spores being plated singly from each irradiated sample and 50 from each control sample. In obtaining macroscopic mutants, both the single spore and dilution plate techniques were used. The lethal mutants were counted and expressed as a percentage of the numbers of germinated spores; the macroscopic mutants were expressed as a percentage of the number of visible colonies.

X-rays were obtained from a Watson Victor S.F.1 dental x-ray unit. Special precautions were taken to accurately locate the x-rays at the distance of 0.45 inch from the focal spot. Calibration figures, supplied by Dr. Eddy of the Commonwealth X-ray and Radium Laboratory, for this x-ray unit, show that the air dose at a distance of 0.45 of an inch from the focal spot is 28,200 roentgen units per minute. Eddy's figure checks fairly well with measurements made with a Victoreen metre borrowed from the Royal Hobart Hospital. It is calculated that the dose required to produce 50 per cent lethal mutants is  $94 \times 10^6$ r. Accuracy is not claimed for the measurements of dose shown in this paper which are given for relative value only. Lea (1946) states that the ionization in tissue is 1000 times that in air.

Soft x-rays were obtained from a Shearer tube unit, which was constructed in this laboratory. Unfortunately no measure of dose was made. The macroscopic mutants which were produced by soft x-ray irradiation were similar to those produced by x-rays from the dental unit.

*The Relation between Dose and Numbers of Lethal Mutants with X-Rays*

Table I shows the relation between dose and numbers of lethal mutants. The first column gives the dose in roentgens, column 2 gives the numbers of single germinated spores, column 3 gives the ratio of the percentage experi-

TABLE I  
Showing Relation between Dose and Numbers of Lethal Mutants with Hard X-Rays

Dose	No. of single germinated spores	Ratio $\frac{\text{Experimental germination}}{\text{Control germination}}$	No. lethal mutants	Lethal mutants
<i>roentgens</i>				<i>per cent</i>
$9.4 \times 10^3$	329	1.02	4	1.21
$18.8 \times 10^3$	373	0.99	3	0.81
$37.6 \times 10^3$	378	0.98	26	6.87
$47.0 \times 10^3$	77	1.6	3	3.89
$56.0 \times 10^3$	302	1.05	45	14.9
$75.2 \times 10^3$	446	0.87	96	21.5
$94.0 \times 10^3$	96	0.95	48	50.0
$112.8 \times 10^3$	62	1.04	38	61.2
$131.0 \times 10^3$	64	1.2	58	90.6
$164.5 \times 10^3$	61	0.96	56	91.63
$188.0 \times 10^3$	55	1.67	47	85.45
$235.0 \times 10^3$	35	1.39	33	94.28
$329.0 \times 10^3$	10	0.57	10	100
Total experi- mental.....	2,189	—	467	21.7
Total controls....	1,353	—	1	0.07

mental germination to control germination, column 4 shows the number of lethal mutants, and column 5 the percentage lethal mutants. A total of 467 mutants was obtained from 2,189 germinated spores. Experiments using a wide range of dose were made extending from  $9.4 \times 10^3$  roentgens, which gave 1.2 per cent lethal mutants, to  $329 \times 10^3$  roentgens which gave 100 per cent lethal mutants. It will be seen that only one (0.07 per cent) lethal mutant was obtained in 1,353 control colonies. Column 3 giving the ratio of experimental to control germination shows that for almost the whole range of dose from  $9.4 \times 10^3$  to  $235 \times 10^3$  roentgens the ratio is approximately constant. The ratio of experimental to control germination is low (0.57 per cent) only at the highest dose used.

Fig. 1 shows the percentage lethal mutants plotted against dose in roentgens. The percentages of lethal mutants are shown as circles on the graph and the standard deviation of each circle or point is given as a vertical line. Theoretical curves for one, two, five, and eight quantum hits are shown on this figure. These curves are taken from Madam Curie's paper (1929). The percentages obtained in the dose-mutant relation experiments made at the ultraviolet wave length 2804 are shown for comparison as crosses in this graph. It will be seen that the x-ray points fit most closely to the five quantum hit curve.

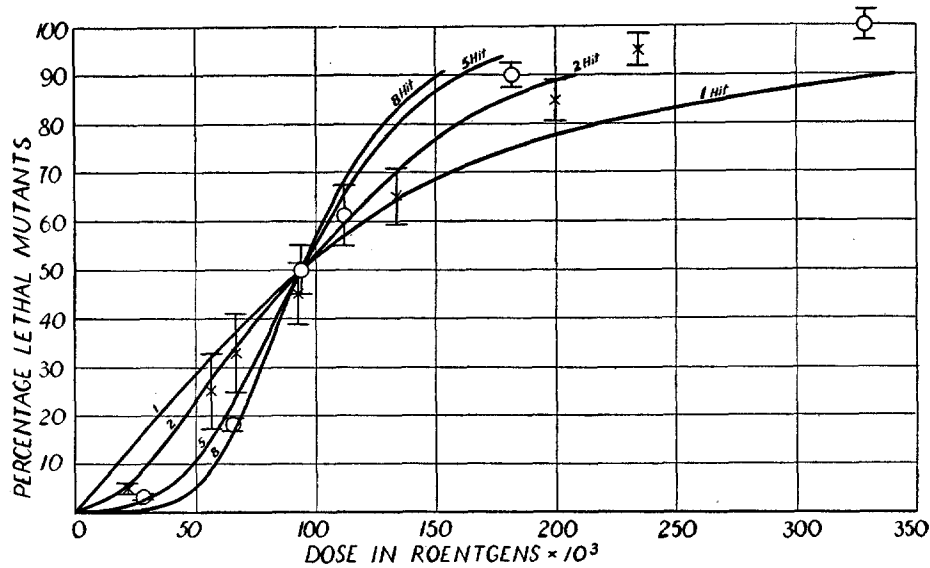


FIG. 1. Showing experimental points for percentage lethal mutants obtained with x-rays from the dental unit plotted against dose in roentgens as circles, and points for the ultraviolet wave length 2804 shown for comparison as crosses. Theoretical curves for one, two, five, and eight quantum hits are plotted.

The points on the most important part of the curve, that is at its beginning, have a very low standard deviation, and the fit to the five quantum hit curve is extremely close. This suggests that five quantum hits are required on the sensitive spot of the spore in order to produce a lethal mutant. The points for the 2804 ultraviolet wave length, which are taken from an unpublished paper by McAulay, Ford, and Dobie, are consistent with a two quantum hit mechanism.

The form of the curves in Fig. 1 suggests that x-rays require more quantum hits to produce a mutant than ultraviolet irradiation. However, as mentioned in the paper by McAulay, Ford, and Dobie, the two quantum hits thought to be consistent with ultraviolet mutant production may be too low owing to the varying depth of the sensitive spot in the spores.

Fig. 1 shows that the one and two quantum hits are quite inconsistent with mutant production by x-rays. Table II gives results plotted in Fig. 1, the standard deviation  $(pq/n)^{\frac{1}{2}}$  being shown in column 4 of the table.

*Production of Macroscopic Mutants by X-Rays and a Comparison with Those Produced by Ultraviolet*

Table III shows results of experiments carried out with soft x-rays, hard x-rays, short ultraviolet wave lengths, and long ultraviolet and visible wave lengths. The average percentage of lethal mutants in experiments shown in the table is between 60 and 74 per cent, the actual figures being listed in row 12 of the table.

TABLE II  
Showing Standard Deviations of Six Average Percentages of Lethal Mutants Obtained with X-Rays from a Dental Unit

$p$	$q$	$n$	$(pq/n)^{\frac{1}{2}}$	Average dose
Average of lethal mutants	$(100 - p)$	No. of germinated spores	Standard deviation	
per cent				roentgens
3.2	96.8	1157	0.517	$28.2 \times 10^3$
18.2	71.8	748	1.32	$65.6 \times 10^3$
50.0	50.0	96	5.1	$94.0 \times 10^3$
61.2	38.8	62	6.23	$112.0 \times 10^3$
90.2	9.8	215	2.42	$182.0 \times 10^3$
100	0	10	3.16	$329.0 \times 10^3$

The most striking feature of the comparison of the macroscopic mutants produced by x-rays with those produced by ultraviolet wave lengths is the almost entire absence of the *K* mutant in x-ray experiments. Large numbers of the *K* mutant (see row 6) are produced by short ultraviolet wave lengths, (also papers by McAulay, Plomley, and Ford, 1945; Ford, 1947; and McAulay and Ford, 1947), but few are produced by other irradiations. At 3132 the shortest wave length included in the long ultraviolet and visible wave lengths, 0.76 per cent *K* mutants are produced. If these are omitted from the long ultraviolet and visible wave lengths (column 4), only 0.64 per cent *K* mutants remain in this group. Only two *K* mutants were produced in all x-ray experiments, one in soft x-rays, and one in hard x-rays, giving a percentage 0.12 *K*'s produced by x-rays. Compared with these low numbers, over 400 (31 per cent) *K* mutants were obtained at short ultraviolet wave lengths. This evidence suggests that the production of the *K* is confined to a limited region of the electromagnetic spectrum.

When the mutants "other than *K*" produced by x-rays, are compared with those produced by ultraviolet and visible irradiation, it is seen that the percent-

ages of mutants in all four groups are similar. They are 7.6 per cent for hard x-rays, 9.9 per cent for soft x-rays, 6.6 per cent for short ultraviolet wave lengths, and 7.2 per cent for long ultraviolet and visible wave lengths. Many of the mutant types produced by x-rays are identical with those produced by ultraviolet irradiation.

TABLE III

*Showing the Macroscopic Mutants Produced by X-Rays and a Comparison Made with Those Produced by Ultraviolet and Visible Irradiation*

	Hard x-rays from a dental unit		Soft x-rays from a Shearer tube		Short ultra- violet wave- lengths from 2482-3025 Å.u.		Long ultraviolet and visible wave- lengths from 3132-4047 Å.u.	
	No.	per cent	No.	per cent	No.	per cent	No.	per cent
1. No. of experiments . . . . .	5		6		13		12	
2. Total experimental colonies . . . . .	588		686		1330		1409	
3. Total control colonies . . . . .	282		285		608		619	
4. Total macroscopic mutants, ex- perimental . . . . .	50	8.5	69	10.06	502	38.4	123	8.7
5. Total macroscopic mutants, con- trol . . . . .	2	0.34	0	0	2	0.19	5	0.80
6. Total <i>K</i> mutants, experimental . . . . .	1	0.102	1	0.14	414	31.1	21	1.4
7. Total <i>K</i> mutants, control . . . . .	0	0	0	0	0	0	0	0
8. Others than <i>K</i> , experimental . . . . .	49	7.6	68	9.91	88	6.6	102	7.2
9. Others than <i>K</i> , control . . . . .	0	0	0	0	0	0	0	0
10. Total pigmented mutants, ex- perimental . . . . .	13	2.2	11	1.6	3	0.22	9	0.63
11. Total pigmented mutants, con- trol . . . . .	1	0.35	0	0	0	0	0	0
12. Average of lethal mutants, <i>per cent.</i>		61.4		72.3		73.3		69.3
13. Ratio Per cent germination, Exp. . . . .		1.2		0.95		0.75		0.50
14. Dose . . . . .	112.81 × 10 <sup>3</sup>		—		0.25-3.0		20.0-1800	
	(roentgens)				(joules/cm. <sup>2</sup> )		(joules/cm. <sup>2</sup> )	

In row 10 the pigmented mutants are shown separated from "other mutants." More are produced in x-ray experiments than in ultraviolet experiments. The mutants classed as "pigmented" include colonies with pink-red pigmented mycelium, green pigmented mycelium, and brown pigmented mycelium. Some of the pigmented mutants were very distinctive, for example red pigmented mutants were obtained in three x-ray experiments with the Shearer tube unit, but were not produced again although a number of experiments were made in an endeavour to do so. The same instability applies to the other pigmented mutants produced by x-ray irradiation.

The ratio of experimental to control germination (row 13) shows that germination is markedly affected at long ultraviolet and visible wave lengths

(ratio 0.5). A little effect on germination (ratio 0.75) is found with short ultraviolet, and no effect is apparent in x-ray irradiation (1.2 and 0.95 ratios).

In row 14 of the table the dose in roentgens required to produce 61.4 per cent lethal mutants with hard x-rays is shown to be  $112.8 \times 10^8$  r. No measure of dose was made in soft x-ray experiments. In columns 3 and 4 the range of dose in joules/cm.<sup>2</sup> for ultraviolet and visible wave lengths is shown.

## SUMMARY

1. Mutants produced by x-irradiation of fungal spores of *Chaetomium globosum* have been compared with those produced by ultraviolet irradiation.
2. The most striking difference between the mutants produced by x-irradiation and ultraviolet irradiation is the absence in x-ray experiments of the K mutant which is produced in large numbers at short ultraviolet wave lengths.
3. A comparison is made of the relation between x-ray dose and numbers of lethal mutants, and the relation between the short ultraviolet wave length 2804 dose and numbers of lethal mutants. Both are compared with theoretical curves for 1, 2, 5, and 8 quantum hits.
4. The production of lethal mutants by x-rays is shown to be consistent with the theoretical curve for five quantum hits on the sensitive spot of the spore, whereas the production of lethal mutants by the ultraviolet wave length 2804 Å.u. is consistent with two quantum hits.

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