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(With Two Plates)

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INTRODUCTION

Quantitative investigation of the lethal action of eight wave lengths below the short-wave limit of ultraviolet irradiation in nature on the unicellular green alga *Chlorella vulgaris* has been reported in two previous papers (Meier, 1932, 1934). A study was made in these papers of the response of the alga to 12 wave lengths of a mercury vapor arc ranging from 2536 A to 3650 A, which was made possible by the use of a fused quartz spectrograph. By means of a crystal quartz spectrograph, the research has now been extended to the lethal effect of 21 wave lengths in the ultraviolet ranging from 2250 A to 3130 A on the same alga.

The spectroscopic manipulations and physical measurements were made by Dr. E. D. McAlister, of the Division of Radiation and Organisms.

I am deeply grateful to Dr. C. G. Abbot, Secretary of the Smithsonian Institution, for his encouragement and assistance in the interpretation of the results of the experiments here reported.

EXPERIMENTAL PROCEDURE

Chlorella vulgaris has proved to be well adapted to this type of research because of the spherical shape of its single green cell and the uniformity and speed with which the cells, multiplying by oval or elliptical spores, cover the surface of an agar plate. This unicellular green alga consists of a spherical cell containing a parietal chromatophore and one easily visible pyrenoid. The diameter of the cell is usually 3μ to 5μ, although some giant cells exceed 10μ. This alga has been maintained in pure culture in my collection for 5 years.

The nutritive solution in which the algae were grown is Detmer ⅓, a modified Knop solution, made up in the following proportions and then diluted to one-third:

Calcium nitrate	1.	gram
Potassium chloride	0.25	"
Magnesium sulfate	0.25	"
Potassium acid phosphate	0.25	"
Ferric chloride	0.002	"
Distilled water	1.	liter

The surface of a glass plate of dimensions 8 x 10 cm was ground so as to retain the agar poured on it. This plate was placed in a large petri dish 15 cm in diameter and after sterilization in the autoclave at 15 pounds pressure for 20 minutes was covered with a layer about 4 mm thick of Detmer ⅓ agar 2 percent and then sterilized a second time.

When the agar had gelled, a heavy suspension of green cells of *Chlorella vulgaris* that had been growing in an Erlenmeyer flask of Detmer ⅓ solution in diffuse light from a north window was poured over the agar in the petri dish. This suspension was allowed to remain on the agar for 24 hours; then the excess was removed. The petri-dish culture was placed in diffuse light from a north window until the surface of the agar was covered with a uniform green growth of algal cells. The glass plate covered with agar and algae was then cut out of the surrounding agar in the petri dish and placed upright in a sterilized closed brass container with a quartz window. A decker was arranged in front of the slit of the spectrograph to permit the exposure of three different portions of the plate for three different time periods.

Previous experimentation (Meier, 1934) has demonstrated that the wave lengths that prove lethal to the green cells of this alga do not affect the culture medium which covers the glass plate in any way that will accelerate or retard the subsequent growth of the algal cells.

The agar plates used in the experiments described here were inoculated September 6, 1934, and irradiated in October and November, as noted in table 1. Those plates irradiated in December were inoculated November 7. Previous experimentation (Meier, 1934) has shown that the difference in the age of the cultures has no apparent effect on the response of this alga to the ultraviolet irradiation.

A quartz mercury arc was used for irradiation of these cultures. The method of making the absolute measurements of the intensity of the lines in the ultraviolet spectrum with the vacuum thermocouple and double monochromator is similar to the one described by Brackett and McAlister (1932).

INTENSITY DATA

The intensity data for plates 39 to 57 are recorded in table 1. Intensities were measured with a thermocouple. The values to be given below represent the average intensities over the areas affecting the algae. A special experiment was planned to test the joint proportionality of the intensity of irradiation and the time of irradiation to

TABLE 1.—*Intensity Data*

Angstroms	October 26 Plates 39-45, intensity: ergs/sec. cm²	November 8 Plates 46-47, 50-52, intensity: ergs/sec. cm²	November 16 Plates 48, 49, 53, intensity: ergs/sec. cm²	December 12 Plates 54-57, intensity: ergs/sec. cm²
2250	200	280	297	297
2300	430	490	519	519
2323	220	220	233	233
2352	600	640	678	678
2378	940	1,050	1,110	1,110
2399	1,000	1,090	1,160	1,160
2447	320	330	350	350
2463	416	429	455	455
2483	2,340	2,400	2,540	2,540
2536	6,800	6,950	7,370	7,350
2576	535	640	678	678
2602	268	320	339	339
2652	5,900	6,100	6,420	6,730
2699	1,310	1,400	1,480	1,480
2753	960	960	1,020	1,020
2804	3,500	3,570	3,800	3,670
2894	1,500	1,620	1,720	1,720
2925	535	545	578	578
2967	4,300	4,250	4,510	4,510
3022	10,100	10,400	11,000	11,000
3130	20,200	20,300	21,500	21,500

the lethal effect. Accordingly, a smaller diaphragm was placed over the lens of the spectrograph so that five plates, plates 58-63, could be irradiated at $\frac{1}{4}$ the original intensity, *i. e.*, the intensity used for irradiation of all the previous plates. By the adjustment of a 20-cm focal length lens in place of a 30-cm lens at the camera end of the spectrograph, a shorter, more intense spectrum was obtained, giving lines three times the intensity of the original spectrum. This very intense spectrum was used to irradiate plates 64-67. The intensity data for the $\frac{1}{4}$ and 3-fold intensities are recorded in table 2, together with the original intensities of December 14.

RESULTS

Decolorized cells appeared in the green plates where the wave lengths of ultraviolet proved to be lethal or radiotoxic. (See pl. 1.) The times of first appearance, *i. e.*, the periods elapsing between irradiation and visibility of the colorless regions, are tabulated for each plate in tables 3, 4, and 5. The exposure times are also shown in minutes and seconds.

TABLE 2.—*Relative Intensity Data*

Angstroms	Original intensity December 12 Plates 54-57, intensity: ergs/sec. cm²	¼ original intensity December 12, 13 Plates 58-63, intensity: ergs/sec. cm²	3 × original intensity December 14 Plates 64-67, intensity: ergs/sec. cm²
2250	297	74	891
2300	519	130	1,557
2323	233	58	699
2352	678	170	2,034
2378	1,110	278	3,330
2399	1,160	289	3,480
2447	350	88	1,050
2463	455	114	1,365
2483	2,540	636	7,620
2536	7,350	1,838	22,050
2576	678	170	2,034
2602	339	85	1,017
2652	6,730	1,683	20,190
2699	1,480	371	4,440
2753	1,020	255	3,060
2804	3,670	916	11,010
2894	1,720	429	5,160
2925	578	145	1,734
2967	4,510	1,126	13,530
3022	11,000	2,756	33,000
3130	21,500	5,380	64,500

PROPORTIONALITY OF RADIOTOXICITY TO THE PRODUCT OF THE INTENSITY AND THE EXPOSURE TIME

In a previous paper (Meier, 1934) the assumption was made that the radiotoxic effect is proportional to the product of the intensity of irradiation and the duration of irradiation. In other words, a 3-minute exposure to x intensity should produce a given radiotoxic effect in the same reaction time as a 1-minute exposure to 3 x intensity; or again expressed differently, the reaction time is inversely proportional to the lethal dose, which is the product of the exposure time by the intensity.

To prove this assumption, experiments were performed with relative intensities of 1, ¼, and 3-fold, as shown in table 2. The results are recorded in tables 3, 4, and 5. As it is difficult to obtain algal plates coated precisely alike over the entire surface, some variation must be expected in observations of the reaction time or period between irradiation time and the first appearance of each radiotoxic region. Table 6 gives representative cases for each of the rays at the relative intensities studied. They show that for a range of 12-fold the proportionality holds within 40 or 50 percent in practically all cases and often much closer, thereby confirming the assumption that the lethal effect, as measured by the reciprocal of the reaction time, is proportional to the product of the exposure time and intensity. (See pl. 2.) As in many photochemical phenomena in the purely physical world, the total effect may depend on the total number of light quanta absorbed by the individual. If this is true, then any algal cell will die when the necessary number of quanta of ultraviolet irradiation have been absorbed.

THE LETHAL RADIOTOXIC THRESHOLD

A study of tables 1 and 3 indicates that the lethal radiotoxic threshold or minimum amount of radiotoxicity required to produce lethal effect for wave length 2250 A lies at 720 seconds for an intensity of 280 ergs/sec. cm^2. Since the radiotoxic effect is proportional to the product of the intensity and the duration of irradiation, then for 1,000 ergs/sec. cm^2 the exposure required for 2250 A may be set as $.280 \times 720 = 202$ seconds. The reciprocal of this threshold value, 202, gives the radiotoxic spectral sensitivity value, which, with the other computations given above, is recorded in table 7. For the sake of convenience the reciprocals are multiplied by 100. In a similar manner the radiotoxic spectral sensitivity value for each wave length was determined, as shown in table 7, and the smooth curve in figure 1 was drawn.

The determination of the lethal radiotoxic threshold factors as given in table 7 and the location of the radiotoxic spectral sensitivity value for each wave length in the radiotoxic spectral sensitivity curve in figure 1 are discussed in the following paragraphs.

2250 A. No lethal region appeared for this wave length at the exposure of 600 seconds. The algae were killed by this ray in two plates at an exposure of 720 seconds, which gives a radiotoxic spectral sensitivity value of .495. The lethal regions, though visible, are not very distinct, and as the intensity of the ray is so small that multiplying by so large a factor as 3.6 gives a doubtful result, it does not seem

TABLE 3.—*Appearance of Radiotoxic Regions*

Exposure time min. sec.	10	20	40		50		1 60				1½ 90		
Plate no.	39, 40	39, 40	56	57	56	57	39, 45	50	51	52	54	55	57
Time of first appearance of radiotoxic regions	No lethal effect	No lethal effect	*6 days* 2652 *19 days* 2536	*8 days* 2652 *25 days* 2536	*6 days* 2652 *19 days* 2536	*8 days* 2652 *25 days* 2536	*21 days* 2536 2652	*13 days* 2536 2652	*12 days* 2536 2652 2804	*4 days* 2536 2652 *12 days* 2804	*6 days* 2536 2652 *25 days* 2804	*3 days* 2652 2804 *8 days* 2536	*5 days* 2536 2652 *25 days* 2804

Exposure time min. sec.	2 120			2¾ 165		3 180		3½ 210		4 240		6 360	8 480
Plate no.	41, 44	51	52	54	55	51	52	54	55	41, 44	53	53	41, 44
Time of first appearance of radiotoxic regions	*2 days* 2652 2804 *3 days* 2536 *6 days* 2699 *7 days* 2483 2752 *17 days* 2894	*8 days* 2536 2652 2804 *14 days* 2699	*4 days* 2536 2652 2804 *5 days* 2576 2699 *9 days* 2752 *12 days* 2894	*6 days* 2536 2652 *8 days* 2804 *19 days* 2483	*3 days* 2652 2804 *5 days* 2536 *19 days* 2483	*8 days* 2536 2652 2804 *13 days* 2483 2699 2752	*4 days* 2536 2652 2804 *5 days* 2576 2699 2894 *13 days* 2483 2752	*6 days* 2536 2652 2804 *7 days* 2483 *25 days* 2699 2752 2894	*2 days* 2536 2652 *3 days* 2804 *7 days* 2483 *25 days* 2699 2752 2894	*2 days* 2536 2652 2804 *3 days* 2483 2699 *4 days* 2752 *6 days* 2576 *11 days* 2894 *19 days* 2378 2399 2602 2967	*2 days* 2536 2652 2804 *3 days* 2699 *6 days* 2483 *19 days* 2378 2399 2576 2752 2894 2967	*2 days* 2483 2536 2652 2804 *3 days* 2699 2752 *7 days* 2894 *19 days* 2378 2399 2576 2967	*2 days* 2483 2536 2652 2699 2804 *3 days* 2752 2894 *5 days* 2967 *6 days* 2576 2602 *7 days* 2378 2399 *24 days* 2323 2352

TABLE 3.—(*Concluded*)

Exposure time min. sec.	10 600	12 720		16 960			20 1,200		32 1,920	64 3,840
Plate no.	53	46	47	42, 43	46	47	46	47	42, 43	42, 43
Time of first appearance of radiotoxic regions	*2 days* 2483 2536 2652 2699 2804 *3 days* 2752 2894 *8 days* 2576 2602 *19 days* 2378 2399 2967	*2 days* 2300 2323 2652 *4 days* 2378 2804 *5 days* 2483 2536 2894 2967 *7 days* 2699 2752 *27 days* 2250 2576 2602	*2 days* 2323 2378 2652 2804 *4 days* 2300 2576 2602 2894 2967 *5 days* 2483 2536 *7 days* 2699 2752 *27 days* 2250	*2 days* 2323 2352 2378 2399 2652 *3 days* 2300 2447 2463 2483 2536 2576 2602 2699 2752 2804 2894 2967 *6 days* 2250 *10 days* 3022	*2 days* 2300 2323 2378 2652 2804 *4 days* 2447 2463 2576 2894 2967 *5 days* 2352 2483 2536 *6 days* 2399 *7 days* 2699 2752 *11 days* 2250 3022	*2 days* 2300 2323 2352 2378 2399 2652 2699 2752 2804 *4 days* 2300 2447 2463 2576 2602 2894 2967 *5 days* 2483 2536 *11 days* 2250 3022	*2 days* 2300 2323 2352 2378 2399 2447 2463 2576 2652 2804 2894 2967 *5 days* 2483 2536 2602 *7 days* 2699 2752 *11 days* 2250 3022	*2 days* 2323 2352 2378 2399 2652 2699 2752 2804 *4 days* 2300 2447 2463 2576 2602 2894 2967 *5 days* 2483 2536 *11 days* 2250 3022	*1 day* 2300 2323 2352 2378 2399 2483 2536 2652 2804 2894 2967 *2 days* 2447 2463 2576 2602 2699 2752 *6 days* 2250 2925 3022	*1 day* 2300 2323 2352 2378 2399 2483 2536 2652 2804 2894 2967 *2 days* 2447 2463 2576 2602 2699 2752 *3 days* 3022 *6 days* 2250 2925 3022

TABLE 4.—*Appearance of Radiotoxic Regions at ¼ Original Intensity*

Exposure time min. / sec.	2 120	3 180	3¾ 225	4 240				6 360		14 840		16 960	56 3,360	64 3,840
Plate no.	58, 59	59	58	58	59	60	61	60	61	60	61	62, 63	62, 63	62, 63
Time of first appearance of radiotoxic regions	No lethal effect	*8 days* 2652 *10 days* 2536	*8 days* 2536 2652	*8 days* 2536 2652	*6 days* 2536 2652 *35 days* 2483 2804	*5 days* 2536 2652	*5 days* 2536 2652	*5 days* 2536 2652 *6 days* 2804	*5 days* 2536 2652 2804	*5 days* 2536 2652 2804 *6 days* 2483 2576 2699 2752 2894	*5 days* 2536 2652 2699 2804 *6 days* 2483 2752 *31 days* 2576	*4 days* 2483 2536 2576 2652 2804 2894 *6 days* 2699 2752 *25 days* 2967	*2 days* 2378 2483 2536 2652 2804 *4 days* 2323 2352 2576 2699 2752 2894 *7 days* 2399 2602 2925 2967	*2 days* 2378 2483 2536 2576 2652 2699 2752 2804 2894 *4 days* 2250 2300 2323 2352 2399 *7 days* 2602 2925 2967

TABLE 5.—*Appearance of Radiotoxic Regions at 3 × Original Intensity*

Exposure time min. / sec.	30	2½ / 150			3½ / 210			5 / 300	
Plate no.	64, 65	64, 65	66	67	64, 65	66	67	66	67
Time of first appearance of radiotoxic regions	*5 days* 2536 2652 2804 *25 days* 2483 2699 2752	*5 days* 2536 2652 2804 *6 days* 2483 2576 2699 2752 *9 days* 2894 2967	*4 days* 2536 2652 *5 days* 2483 *6 days* 2699 2752 2804 *9 days* 2894 2967	*4 days* 2483 2536 2576 2602 2652 2699 2752 2804 2894 2967	*4 days* 2536 2652 2804 *5 days* 2576 2602 2699 2752 *6 days* 2483 2894 *8 days* 2378 2399 *9 days* 2967	*4 days* 2536 2652 *5 days* 2483 2699 2752 2804 *10 days* 2894 2967	*4 days* 2483 2536 2576 2602 2652 2699 2752 2804 2894 2967	*4 days* 2536 2652 2804 *5 days* 2483 2699 2752 *6 days* 2576 2602 2894 2967 *29 days* 2378 2399	*4 days* 2323 2378 2399 2483 2536 2576 2602 2652 2699 2752 2804 2894 2967 *5 days* 2352

TABLE 6.—*Proportionality of Radiotoxic Effect to Intensity and Irradiation Time*

A	Relative intensity	Plate no.	Exposure time, sec.	×	Intensity	×	Days	=	Product ÷ 10
2250	1	42	960		.200		6		115
	¼	62	3,840		.074		4		114
2300	1	47	720		.490		4		141
	¼	62	3,840		.130		4		200
2323	1	42	3,840		.220		1		845
	¼	62	3,360		.058		4		780
	3	67	300		.699		4		839
2352	1	46	960		.640		5		307
	¼	62	3,840		.170		4		261
	3	67	300		2.034		5		305
2378	1	41	480		.940		7		316
	¼	62	3,840		.278		2		214
	3	67	300		3.330		4		400
2399	1	53	240		1.160		19		529
	¼	62	3,840		.289		4		444
	3	67	300		3.480		4		418
2483	1	54	210		2.540		7		373
	¼	62	3,840		.636		2		488
	3	67	150		7.620		4		457
2536	1	57	90		7.350		5		331
	¼	59	180		1.838		10		331
	3	64	30		22.050		5		331
2576	1	41	240		.535		6		77.0
	¼	60	840		.170		6		85.7
	3	67	150		2.034		4		122
2602	1	53	600		.339		8		163
	¼	62	3,360		.085		7		200
	3	66	300		1.017		6		183
2652	1	57	50		6.730		8		269
	¼	58	225		1.683		8		303
	3	64	30		20.190		5		303
2699	1	52	180		1.400		5		126
	¼	60	840		.371		6		187
	3	67	150		4.440		4		266
2753	1	53	360		1.020		3		110
	¼	60	840		.255		6		129
	3	67	150		3.060		4		184
2804	1	52	120		3.570		4		171
	¼	61	360		.916		5		165
	3	64	30		11.010		5		165
2894	1	52	120		1.620		12		233
	¼	60	840		.429		6		216
	3	67	150		5.160		4		310
2967	1	41	240		4.300		19		1961
	¼	62	960		1.126		25		2702
	3	64	150		13.530		9		1825

that much weight should be given to this point. Therefore, in drawing the smooth curve, a questionable value of .380 is ascribed to this wave length.

TABLE 7.—*Lethal Radiotoxic Threshold and Radiotoxic Spectral Sensitivity*

A	Intensity ergs/sec. cm²	Lethal radiotoxic threshold For given intensity, sec.	Lethal radiotoxic threshold For 1,000 ergs/sec. cm², sec.	Radiotoxic spectral sensitivity Reciprocals × 100	Smooth curve
2250	280	720	202	.495	.380
2300	490	720	353	.283	
	490	650	319	.313	.313
2323	220	480	106	.943	
	699[a]	300	210	.476	
	699	400	280	.357	.357
2352	600	480	288	.347	.404
2378	940	240	226	.442	.442
2399	1,000	240	240	.417	.417
2447	320	960	307	.326	.326
2463	416	960	399	.251	
	416	725	302	.331	
				.291[b]	.300
2483	2,340	120	281	.356	
	2,540	165	419	.239	
				.298[c]	.305
2536	7,350	40	294	.340	
	7,350	31	228	.439	.439
2576	678	240	163	.613	.613
2602	268	240	64	1.563	
	1,017[a]	150	153	.654	.654
2652	6,730	40	269	.372	
	6,730	30	202	.495	.650
2699	1,310	120	157	.637	.625
2753	960	180	173	.578	.578
2804	3,570	60	214	.467	.505
2894	1,500	180	270	.370	.350
2925	535	1,920	1,027	.097	
	535	1,560	835	.120	.200
2967	4,300	240	1,032	.097	.097
3022	10,100	960	9,696	.010	.010
3130	20,200				

[a] See plate 67. [b] Mean of .251 and .331. [c] Mean of .356 and .239.

2300 A. No algae were killed at 2300 A by an exposure of 600 seconds. Lethal effect appears for the first time with one exposure at 720 seconds, which gives a radiotoxic spectral sensitivity value of .283. If it is assumed that there is lethal effect at 650 seconds' exposure, the lethal factor becomes .313.

2323 A. This ray produced a lethal effect with an exposure of 480 seconds, which gives a radiotoxic sensitivity value of .943. This value seems too high. In the 3-fold intensity experiment, 2323 A was lethal with 300 seconds' exposure on one plate, but not on any other plate, which would seem to indicate that the threshold is higher than 300 seconds. If an exposure time of 400 seconds is selected, a radiotoxic spectral sensitivity value of .357 is obtained.

2352 A. Algal cells were first killed by this ray at an exposure of 480 seconds, which gives a radiotoxic spectral sensitivity value of .347 and a value of .404 on the smooth curve.

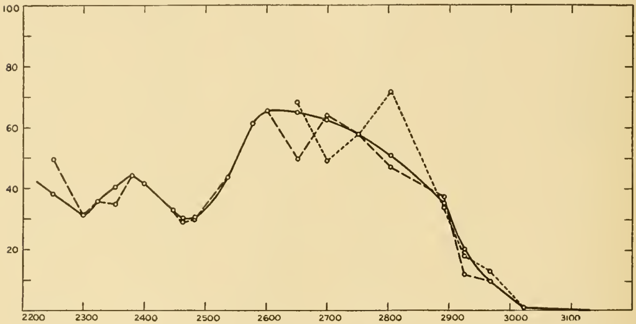

Fig. 1.—Radiotoxic spectral sensitivity of *Chlorella vulgaris* to ultraviolet rays. The abscissae are wave lengths in angstroms. The ordinates are relative lethal effectiveness in arbitrary units. Black line, smooth curve; dash line, actual values; dot line, curve obtained by Meier (1934).

2378 A. There was lethal action by this ray with an exposure of 240 seconds, which gives a radiotoxic spectral sensitivity value of .442.

2399 A. The lethal threshold of this ray is at 240 seconds' exposure, thus giving a radiotoxic spectral sensitivity value of .417.

2447 A. A lethal effect is first produced by this ray in the algal plates exposed for 960 seconds. The radiotoxic spectral sensitivity value is .326.

2463 A. On the algal plates, lethal effect was observed with an exposure of 960 seconds at two slightly different intensities, namely, 416 and 429 ergs/sec. cm^2. The lethal factor for 960 seconds at 416 ergs/sec. cm^2 is .251. The exposure lower than 960 seconds was 720 seconds, and although there was no lethal effect on the algae at one 720-second exposure, it seems very possible, considering the intensity of the line, that there might be lethality at 725 seconds, thereby raising the radiotoxic spectral sensitivity value to .331. The mean of these two values is .291, which gives a reasonable value of .300 on the smooth curve.

2483 A. The algae were killed by this ray on two plates, but not on two others at 120 seconds' exposure, which would give a radiotoxic spectral sensitivity value of .356, which seems too high when plotted. At an exposure of 165 seconds and at higher exposures, the algae were always killed by this ray. If 165 seconds' exposure were considered as the threshold, the radiotoxic spectral sensitivity value would become .239, which seems too low in comparison with the intensity of the line. However, the mean of the two values seems reasonable, and on the smooth curve the point becomes .305.

2536 A. This ray proved lethal at an exposure of 40 seconds, which gives a radiotoxic spectral sensitivity value of .340. There was no lethal effect with the 20 seconds' exposure, but as the intensity of the ray is very high, there possibly would have been lethal effect at 31 seconds, which gives a radiotoxic spectral sensitivity value of .439.

2576 A. The lethal threshold for this wave length apparently lies at 240 seconds, which gives a radiotoxic spectral sensitivity value of .613. Lethal regions for this ray are perceived on one algal plate at 120 seconds' exposure, but not on five others; again on one plate at 180 seconds' exposure, but not on another at the same exposure; and there were no lethal regions on the three plates made at an exposure of 210 seconds. It is only at 240 seconds' exposure and higher that this lethal region appears with certainty.

2602 A. If the exposure of 240 seconds is considered the lethal threshold of this ray, the radiotoxic spectral sensitivity value becomes 1.563, which is unreasonably high. In the experiment with 3-fold intensity the threshold is at 150 seconds, which gives a more reasonable radiotoxic spectral sensitivity value of .654.

2652 A. The lethal threshold for this ray on the algal plates was 40 seconds, which gives a radiotoxic spectral sensitivity value of .372. Considering the high intensity of this ray, a very large divisor (6.7) was used to reduce it to the standard intensity of 1,000. Therefore this point has small weight and is probably too low. It is possible that lethal effect might have been produced at 30 seconds, since the 20 seconds' exposure showed no lethal effect. In that case the radiotoxic spectral sensitivity value would be .495, and .650 on the smooth curve.

2699 A. 120 seconds' exposure to this ray produced lethal effects in four different plates, giving a radiotoxic spectral sensitivity value of .637, which is lowered to .625 on the smooth curve.

2753 A. This ray proved lethal to the algae in three plates at an exposure of 120 seconds, but no lethal regions for this ray appeared in the plates exposed for 165 seconds. Consequently, 180 seconds seems to mark the threshold of lethal action for 2753 A, thus giving a radiotoxic spectral sensitivity value of .578.

2804 A. An exposure of 60 seconds at an intensity of 3,570 ergs/sec. cm^2 produced lethal action, whereas the same exposure at 3,500 ergs/sec. cm^2 did not. Consequently, the first-named exposure seems to mark the threshold, giving a radiotoxic spectral sensitivity value of .467, which on the smooth curve becomes .505.

2894 A. Lethal regions for this ray first began to appear at an exposure of 120 seconds, but as they did not appear on the plates at an exposure of 165 seconds, the threshold seems to be in the vicinity of 180 seconds, which gives a radiotoxic spectral sensitivity value of .370, or .350 on the smooth curve.

2925 A. This ray, which is of very weak lethal value, did not kill the algae at an exposure of 1,200 seconds, but did at an exposure of 1,920 seconds, which would give it a radiotoxic spectral sensitivity value of .097. It seems more likely that the threshold would be midway between 1,200 and 1,920 seconds, or at 1,560 seconds, which would give a radiotoxic spectral sensitivity value of .120 or .200 on the smooth curve.

2967 A. The lethal threshold for this ray is at an exposure of 240 seconds, giving a radiotoxic spectral sensitivity value of .097.

3022 A. A lethal region for this ray is faintly visible at 960 seconds' exposure, thus giving a radiotoxic spectral sensitivity value of .010.

3130 A. This ray did not produce a lethal effect even in the experiment with 3-fold intensity.

The radiotoxic spectral sensitivity values of the curve previously determined by Meier (1934) when reduced to the same scale show reasonable agreement with the new values for the radio-toxic spectral sensitivity smooth curve as may be seen in figure 1.

RADIOTOXIC SPECTRAL SENSITIVITY AND RADIOTOXIC VIRULENCE

As described in a previous paper, the lethal response of the algae to the ultraviolet rays may be considered from two points of view: as to the radiotoxic spectral sensitivity and the radiotoxic virulence. The term " radiotoxic spectral sensitivity " relates to the certainty of the lethal action, while the term " radiotoxic virulence " may be used to describe the speed of the attack.

The determination of the radiotoxic spectral sensitivity, that is, the relative radiotoxicity of rays of different wave lengths when applied with equal intensity and duration, has been measured as described in the preceding section. The determinations for each of the ultraviolet rays when plotted against wave lengths give a curve of radiotoxic spectral sensitivity. (See table 7 and fig. 1).

The radiotoxic virulence or the determination of the speed with which the toxic doses of the different rays produce lethal effects has been made by computing the brevity of time required to produce lethal effect for a standard radiotoxic quotum exceeding the lethal radiotoxic threshold.

A number of exposures in which many lethal wave length regions appeared were selected for determination of the radiotoxic virulence. The first appearances of these lethal regions were dispersed over long periods. The radiotoxic quotum, or the amount of radiotoxicity applied, is apparently proportional to (1) the exposure time, (2) the intensity of the ray, and (3) the radiotoxic spectral sensitivity. The radiotoxic virulence is, however, inversely proportional to (1) the radiotoxic quotum applied, and (2) the reaction time. Determinations of the virulence values for radiotoxic regions of 20 wave lengths with exposure times varying from 2 to 32 minutes were made as shown by the following example, which includes the computations for the wave length 2463 A.

A	Plate no.	Exposure time, min. ×	Intensity ×	Radiotoxic spectral sensitivity =	Radiotoxic quotum ×	Days =	Product	Reciprocals proportional to virulence
2463	42	16	4.16	3.00	199.8	3	599.4	.00167
	46	16	4.29	3.00	205.8	4	825.6	.00121
	47	16	4.29	3.00	205.8	4	825.6	.00121
	46	20	4.29	3.00	257.4	2	514.8	.00194
	47	20	4.29	3.00	257.4	4	1,029.6	.00097
	42	32	4.16	3.00	399.3	2	798.6	.00125

The virulence values for each exposure were then plotted against the wave lengths.

As the curve resulting from the 32-minute exposure on plate 42 included all 20 of the radiotoxic regions and appeared typical, it was chosen as a standard. The 12 other curves were then reduced to the same scale as the standard, 42 (32), in the following manner. First, each value of 42 (32) was divided by each corresponding value of the 12 other exposures. For example, the computations for 2463 A would be:

Plate no.	42	42	46	47	46	47
Exposure time	32	16	16	16	20	20
2463 A	1.00	0.75	1.03	1.03	0.64	1.29

The last preceding line results from dividing the numbers marked "reciprocals" above into .00125, the reciprocal corresponding to 42 (32).

In a similar manner the computations were made for all 20 wave lengths. After the widely divergent values had been discarded, the mean of all the values of each exposure was then determined, including all the observed wave lengths found on that exposure. This quantity is called "average multiplier" in the illustration below. Secondly, the products were found of each original virulence value multiplied by the corresponding mean value for each exposure, as shown below by continuing with the illustration of the wave length 2463 A.

Plate no.	42	42	46	47	46	47		
Exposure time	32	16	16	16	20	20		
Average multiplier	1.00	1.06	1.51	1.29	1.59	1.69	Sum	Virulence mean
2463 A (x 100)	.125	.177	.183	.156	.308[a]	.164	.805	.161

[a] This value, being wild, is rejected in the mean.

The mean for each wave length resulting from these final computations constitutes the general result of the whole research on virulence for each wave length, and each of the virulence values as plotted against the corresponding wave length gives the curve of radiotoxic virulence shown in figure 2.

The average deviation of the individual values of virulence from the mean was next determined in the following manner. As shown in the illustration given below, the difference of the final virulence mean from the individual mean value for each exposure of the individual wave lengths was computed.

							Average deviation	Average deviation percent	Probable error percent
Plate no.	42	42	46	47	46	47			
Exposure time	32	16	16	16	20	20			
2463 A (deviation)	36	16	22	5	147[a]	3	16.4	10.3	3.9

[a] This value, being wild, is rejected in the mean.

The mean of these differences for each wave length constitutes the average deviation. The percentage average deviation was then computed by dividing the average deviation for each wave length by the corresponding virulence mean. The percentage probable error was next computed according to the formula:

$$\frac{0.84 \text{ percentage average deviation}}{\sqrt{n-1}}$$

where n = the number of observations. The percentage probable error is illustrated in figure 2.

DISCUSSION

The radiotoxic spectral sensitivity curve for the green alga *Chlorella vulgaris* in figure 1 is of the same general type in the region 2600 to 3130 A as the curves representing bactericidal action and virus inactivation as found by Duggar and Hollaender (1934); the lethal effect on yeast by Oster (1934); the lethal effect on paramoecium by Weinstein (1930); and bactericidal action and protein coagulation by Sonne (1928), and Rivers and Gates (1928).

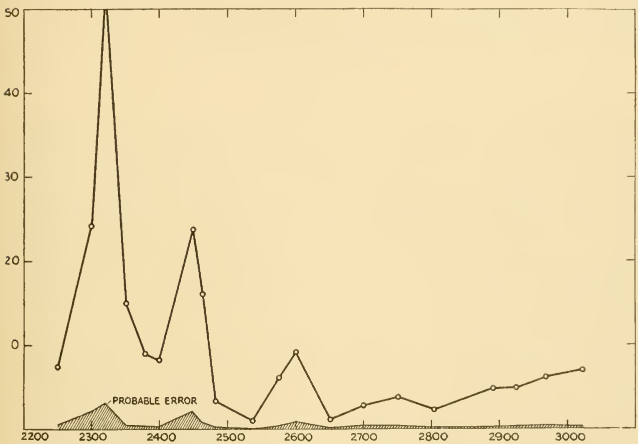

FIG. 2.—Radiotoxic virulence determined from *Chlorella vulgaris*. The abscissae are wave lengths in angstroms. The ordinates are radiotoxic virulence in arbitrary units.

Very little study has been made of the effect of wave lengths of the ultraviolet below 2600 A on algae. Lucas (1934) has given us an idea of the actual effect of an ultraviolet ray on an individual algal cell. By means of a specially designed ultraviolet microscope with a monochromatic system, Lucas has photographed living cells of the blue-green alga *Gloeocapsa*. Wave lengths 2573 A and 2750 A showed good definition of the cells, although the absorption of the cell increased at 2573 A. At 2300 A, the absorption of the cell was very strong, but the photographs were not sharp or distinct. No photographs were possible with wave length 2265 A, as, for a second or two while under visible observation with the fluorescent ocular, the cells seemed agitated or strained and then suddenly increased to 5 or 10 times their original diameter. This distortion was followed by shrinkage and finally disintegration. Since wave length 2750 A did not affect the cells, Lucas studied the reactions to wave length 2300 A by photographing one specimen with wave length 2750 A, irradiating it for a given time with wave length 2300 A, and then rephotographing with wave length 2750 A. By irradiating and photographing in regular sequence, Lucas obtained a series of photographs showing the cumulative effects of irradiation. At 2300 A, the cells appeared quite opaque, and when the irradiation time was increased for this wave length, separation of the different cell parts occurred. At 2265 A there was also general disintegration of the cells.

SUMMARY

The radiotoxic spectral sensitivity of the unicellular green alga *Chlorella vulgaris* has been determined for 20 wave lengths in the ultraviolet ranging from 2250 A to 3022 A. As a preliminary step to facilitate deductions, an experiment was performed with three relative intensities which demonstrated that for a range of 12-fold the lethal effect as measured by the reciprocal of the reaction time is approximately proportional to the product of the exposure time and intensity. The results are in good accord with earlier observations as far as those went. They show maximum lethal sensitivity at about 2600 A. The wave length 3130 A, slightly longer than the short wave length limit of solar radiation reaching the earth's surface, had no lethal effect on the algal cells although of a greatly higher intensity than the toxic wave lengths. Since it is true that death ensued more quickly in some regions than in others, the radiotoxic virulence, or speed of effectiveness of each lethal ray in killing the algal cells for a radiotoxic quotum has been calculated for 20 wave lengths ranging from 2250 A to 3022 A. The results are of small percentage probable error, and they show an interesting wave of virulence alternately waxing and waning with decreasing wave length, but reaching a high maximum at 2323 A.

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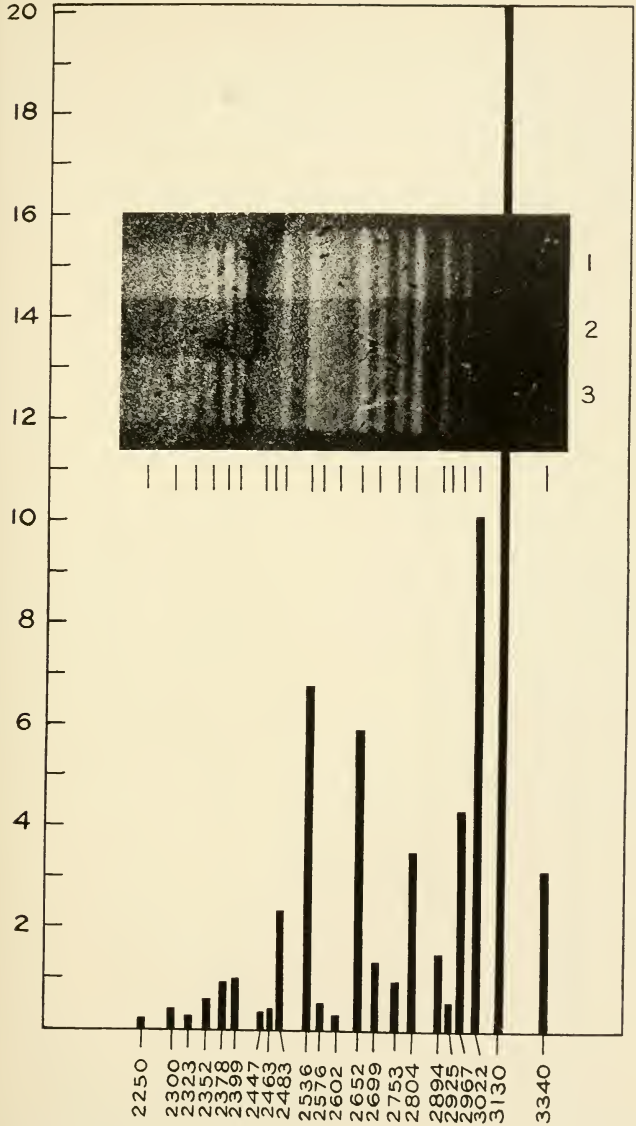
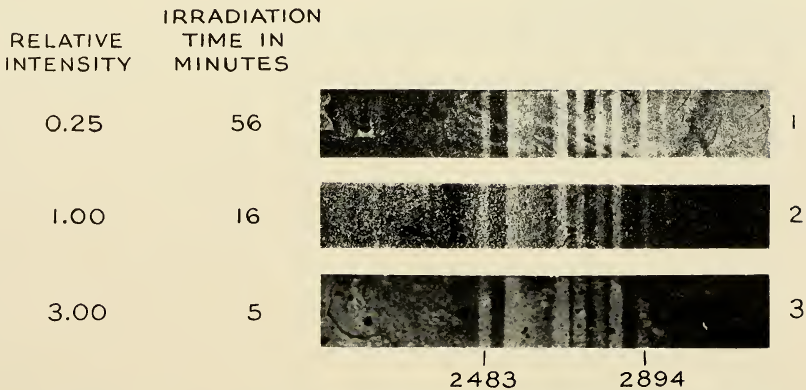


An algal spectrogram, obtained by exposing plate 42 of *Chlorella vulgaris* to ultraviolet rays for (1) 64 minutes, (2) 16 minutes, and (3) 32 minutes, superimposed on a diagram of the intensities of the wave lengths.

The abscissae are wave lengths in angstroms; the ordinates are intensities in thousands of ergs/sec.cm².



Radiotoxic regions in (1) plate 62, (2) plate 42, (3) plate 67 of *Chlorella vulgaris* exposed to relative intensities of ultraviolet rays. The lethal regions on all three plates were visible about 4 days after irradiation.