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INCREASED STIMULATION OF THE ALGA STICHOCOCCUS BACILLARIS BY SUCCESSIVE EXPOSURES TO SHORT WAVE LENGTHS OF THE ULTRAVIOLET

(With Two Plates)

BY
FLORENCE MEIER CHASE
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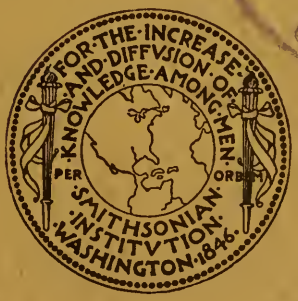

(Publication 3603)

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INTRODUCTION

The stimulation of growth as measured by cell multiplication in the green alga *Stichococcus bacillaris* Naegeli has been produced by the optimum stimulative exposure of the cells to each of four short wave lengths of the ultraviolet. The optimum stimulative exposure occurs at approximately two-thirds of the lethal threshold of each of the wave lengths 2352, 2483, 2652, and 2967 A. (Meier, 1939). Since the stimulative action is not transitory but has persisted in the cells for the past 3 years, and since the stimulated cells, though slightly shorter and wider than the control cells, are also greener and in better condition, further research was undertaken, as described here, to determine the effect of successive stimulative exposures of the algal cells to each of the above-mentioned wave lengths. Irradiations of the stimulated cells and control cells were also made to ascertain the difference in their sensitivity to the lethal wave lengths of the ultraviolet spectrum.

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

It gives me great pleasure to express my appreciation to Dr. C. G. Abbot, Secretary of the Smithsonian Institution, for his advice given continuously during the course of these investigations. I am grateful to Mr. John A. Roebling for his suggestive query regarding the lethal sensitivity of the stimulated cells. I wish to thank Dr. E. S. Johnston and the other members of the Division of Radiation and Organisms for their cooperation in this research.

LITERATURE

The investigations by other workers on the lethal and stimulative effect of the ultraviolet have been reviewed in previous papers (Meier, 1934, 1936, 1939).

I. SUCCESSIVE STIMULATION

EXPERIMENTAL PROCEDURE

A quartz mercury-vapor arc and a fused quartz prism spectrograph were used for the exposures to the wave lengths 2652 and 2967 A. A spectrograph with crystal quartz prisms served for the irradiations with the wave lengths 2352 and 2483 A. Absolute measurements of the intensity of the lines were made with a Clark vacuum thermocouple as described by Johnston and Weintraub (1939) and a double monochromator as in the method described by Brackett and McAlister (1932).

The method used for the growth and irradiation of the algal cultures has already been described in the previous paper (Meier, 1939), but for the sake of clearness and convenience it is briefly repeated here.

The unicellular green alga *Stichococcus bacillaris* Naegeli lends itself satisfactorily to precise and accurate counting and measurement because of its size and method of multiplication. This alga has an elongated cell usually varying from 1 to 2μ in width and 4 to 8μ in length. Multiplication takes place by transverse division of the protoplast that partially fills the cell and by the formation of cross walls, thus developing two cells in place of the one parent cell. The nucleus usually lies near the center of the cell. Filaments of more than two cells were rarely observed in the cultures. The alga develops rapidly, forming a green deposit in Detmer 1/3 solution.

The nutritive solution Detmer 1/3, which was used entirely for this series of experiments, was made up in the following proportions and then diluted 1/3:

Calcium nitrate	1.0	gram
Potassium chloride	0.25	"
Magnesium sulfate	0.25	"
Potassium acid phosphate	0.25	"
Ferric chloride	0.002	"
Distilled water	1.0	liter

Before irradiation, algal cells were pipetted from actively growing cultures into small quartz tubes, which were designed and constructed by L. B. Clark, of the Division of Radiation and Organisms. One side of each tube was flattened so as to insure equal and complete irradiation of the contents. Each quartz tube was equipped with a slender stirrer made of nichrome wire No. 24 inserted through the cork so that the culture could be stirred during irradiation. After the stemlike base of the tube had been securely inserted in a rubber

stopper so placed as to hold the tube directly in the monochromatic ultraviolet ray, the tube was examined with a piece of uranium glass to insure that the contents were covered by the ultraviolet ray. The quartz tube transmitted approximately 90 percent of the ultraviolet ray. A separate quartz tube was used for each exposure. Thermocouple measurements of the intensity were made before and after each experiment. The ultraviolet lamp was turned on half an hour before each experiment so that the intensity of the radiation was constant when the thermocouple measurements were made. The control cultures were treated exactly in the same manner as the irradiated cultures except that they were not exposed to the ultraviolet.

After irradiation, the contents of each tube were pipetted into a 300-cc. Erlenmeyer flask containing 200 cc. of Detmer 1/3, which had been sterilized in the autoclave at 20 pounds pressure for 15 minutes. After being thoroughly agitated, 100 cc. of the culture was poured into a second 300-cc. Erlenmeyer flask so that duplicate cultures were obtained for each exposure. The flasks were equipped with rubber stoppers, which were found to be more satisfactory than cotton plugs, previous experimentation having shown that the algae grow equally well in the rubber-stoppered flask and in the flask with a cotton plug, provided the cultures were inoculated a week or more after the flasks of culture medium had been autoclaved.

The cells of three drops of the culture from each flask were counted directly after irradiation, and the mean of the three cell counts was taken as the initial cell count. The pipette used for making the drops for the initial count was marked, cleaned with ether, and put away for use with the same culture 2 weeks later when the final count was made in similar fashion to the initial count. A separate pipette was assigned to each flask. In this manner equal size drops were obtained from each culture. The quartz irradiation tube contained generally 24 drops of inoculum, which were divided between the two Erlenmeyer flasks in the manner described above. The number of cells per drop of inoculum for each culture of the same experiment was fairly uniform. The number of cells per drop of inoculum varied in the different experiments.

To insure counting every cell in a drop, a special microscope slide was etched for the purpose by L. A. Fillmen, of the Division of Radiation and Organisms, in the following manner: The slide was coated with a thin layer of beeswax and then ruled into rectangles on the milling machine with a sharp-pointed tool. The lines were 1 mm. apart lengthwise and 4 mm. apart crosswise. The lines were

etched into the glass by placing a drop of hydrofluoric acid with a glass rod on the slide, and by spreading the drop with the glass rod into the grooves where it rested for a fraction of a minute. The acid was washed off with water, the beeswax was scraped off with a sharp flat tool, and the slide was cleaned.

The special pipettes made by L. B. Clark were drawn to a point so that a drop from each could be covered completely by a No. 2 A, $\frac{3}{4}$-inch cover glass. By using a euscope attachment to the microscope and a mechanical stage, it was a simple matter to count every cell on the slide with either the high-power or the low-power objective and a No. 5 ocular.

To arrange an ideal environment for the growth of the cultures after irradiation, an electric refrigerator was equipped with a special thermostat, which held the temperature at 24° C. during the light period and 22° C. during the dark period. Fluorescent daylight lamps were tested and proved to produce better growth conditions for the algae than varying daylight. A set of four of these 15-watt daylight lamps was installed. The lights gave an intensity of approximately 150 foot-candles on the bottom shelf and 300 foot-candles on the top shelf.

The flasks of algae were placed on the lower shelf of this chamber and illuminated for 12 hours of each 24 hours during their growth period of 2 weeks.

In the present work, cultures in which the growth of the cells had been previously stimulated by an exposure to the ultraviolet were given repeated exposures at intervals of a month or more to the same wave length. The final growth ratios are presented in tables 1, 2, 3, and 4. Since the agreement between the duplicate cultures of the individual experiments was in the same order as illustrated in table 1 which shows the complete results obtained with 2352 A., in an effort to save space, the means of the results of the duplicate cultures are given for the three other wave lengths in tables 2, 3, and 4.

To make tables 1, 2, 3, and 4 perfectly clear, the following example is given: From a control culture of algae that had been irradiated in May 1937 (see table 1), a quartz tube of algal cells was drawn for the experiment of May 1939 and then pipetted into 200 cc. of newly made and autoclaved Detmer 1/3 solution in a 300-cc. Erlenmeyer flask. After the contents of the flask had been agitated, 100 cc. of the culture were poured into a second 300-cc. Erlenmeyer flask. Cells from a culture that had been irradiated 12 minutes (the optimum stimulation time for 2352 A.) in May 1937 were poured

into a second quartz tube and irradiated for 12 minutes by 2352 A., then the cells were placed in 200 cc. of Detmer 1/3 in a 300-cc. Erlenmeyer flask and after agitation the contents were divided into a second flask. The cultures for the experiment of August 1939 were inoculated from the cultures of the experiment of May 1939, and those for the experiment of February 1940 from the ones of the experiment of August 1939. As described above, duplicate cultures were made in each instance.

TABLE 1.—*Increased stimulated growth*[1] *resulting from repeated exposures to 2352 A.*

Exposures (minutes)				Growth ratios $\frac{S}{C}$			
May 1937 [590][2]	May 1939 [608]	Aug. 1939 [605]	Feb. 1940 [600]	May 1937	May 1939	Aug. 1939	Feb. 1940
0	0	0	0	1.0(2.6)[3]	1.0(1.4)	1.0(1.5)	1.0(1.4)
0	0	0	0	1.0	1.0	1.0	1.0
12	.....	.....	.....	1.7	1.6	1.6	.....
12	.....	.....	.....	1.8	1.5	1.4	.....
.....	12	.....	.....	.....	1.3	1.5	.....
.....	12	.....	.....	.....	1.4	1.6	.....
.....	12	12	.....	.....	1.9	3.1	.....
.....	12	12	.....	.....	1.9	2.9	.....
12	12	12	.....	.....	.....	3.3	.....
12	12	12	.....	.....	.....	3.3	.....
.....	12	12	12	.....	.....	3.4	3.9
.....	12	12	12	.....	.....	3.8	3.6
12	12	12	12	.....	.....	.....	4.6
12	12	12	12	.....	.....	.....	4.8

[1] Growth is measured here by cell multiplication.
[2] Figures in brackets = intensity in ergs/sec.cm.[2]
[3] Figures in parentheses = growth rate of control cultures in each experiment.

TABLE 2.—*Increased stimulated growth*[1] *resulting from repeated exposures to 2483 A.*

Exposures (seconds)				Growth ratios $\frac{S}{C}$			
May 1939 [2620][2]	Nov. 1939 [2520]	Jan. 1940 [2480]	Mar. 1940 [2420]	May 1939	Nov. 1939	Jan. 1940	Mar. 1940
0	0	0	0	1.00(1.2)[3]	1.00(1.5)	1.00(1.8)	1.00(1.5)
30	.....	.....	.....	1.70	.....	.....	.....
.....	30	.....	.....	.....	1.60	.....	.....
30	40	.....	.....	.....	2.45	.....	.....
.....	30	30	.....	.....	.....	1.80	.....
30	30	30	.....	.....	.....	2.60	.....
.....	30	30	30	.....	.....	.....	3.16
30	30	30	30	.....	.....	.....	3.90

[1] Growth is measured here by cell multiplication.
[2] Figures in brackets = intensity in ergs/sec.cm.[2]
[3] Figures in parentheses = growth rate of control cultures in each experiment.

TABLE 3.—*Increased stimulated growth*[1] *resulting from repeated exposures to 2652 A.*

Exposures (seconds)				Growth ratios $\frac{S}{C}$			
Dec. 1938 [1980][2]	Nov. 1939 [1970]	Dec. 1939 [1940]	Mar. 1940 [1960]	Dec. 1938	Nov. 1939	Dec. 1939	Mar. 1940
0	0	0	0	1.00(1.6)[3]	1.00(1.5)	1.00(2.6)	1.00(1.3)
40	.....	.....	.....	1.80	.....	.....	.....
.....	40	.....	.....	.....	1.70	.....	.....
40	40	.....	.....	.....	2.25	.....	.....
.....	40	40	.....	.....	.....	2.10	.....
40	40	40	.....	.....	.....	3.15	.....
.....	40	40	40	.....	.....	.....	2.35
40	40	40	40	.....	.....	.....	4.65

[1] Growth is measured here by cell multiplication.
[2] Figures in brackets =intensity in ergs/sec.cm.[2]
[3] Figures in parentheses =growth rate of control cultures in each experiment.

TABLE 4.—*Growth*[1] *resulting from repeated exposures to 2967 A.*

Exposures (seconds)		Growth ratios $\frac{S}{C}$	
Feb. 1939 [2370][2]	Sept. 1939 [2330]	Feb. 1939	Sept. 1939
0	0	1.00(1.5)[3]	1.00(1.5)
200	.....	1.75	1.55
.....	200	.....	1.55
200	200	.....	1.10

[1] Growth is measured here by cell multiplication.
[2] Figures in brackets =intensity in ergs/sec.cm.[2]
[3] Figures in parentheses =growth rate of control cultures in each experiment.

RESULTS

After four successive irradiations, the cells exposed to 2352 A. were stimulated to a growth rate of 4.7 times that of the control; those exposed to 2483 A. to 3.9 times the control and those exposed to 2652 A. to 4.65 times that of the control. Although the cells were stimulated by the first irradiation with 2967 A. to 1.62 times the control, they did not respond to the second irradiation and their rate of increase was practically the same as that of the control.

CELL MEASUREMENTS

The lengths and widths of 500 cells in a representative culture of each set of irradiated cultures, and the controls, were measured with an ocular micrometer. From these data the means of the measurements were computed and tabulated in table 5. The ratios were computed by dividing the mean length for 500 cells of the stimulated

cultures by the mean length of 500 cells of the controls. The ratios for the width were obtained in a similar manner.

A study of the table shows that the cells decrease in length with each stimulative exposure to the ultraviolet. The cells increase in width except with the final fourth exposures of 2352 A. and 2483 A. The decrease in length is to be expected since the rate of the multiplication of cells in the stimulated cultures is so much higher than in the controls that the stimulated cells do not have time to attain the length found under normal conditions before they divide to form new cells.

TABLE 5.—*Cell measurements* [1]

A.	Exposure	Mean length μ	Ratio $\frac{S}{C}$ Percent	Mean width μ	Ratio $\frac{S}{C}$ Percent
	Minutes				
2352	0	4.717	.....	1.184	.....
	12	4.326	91.7	1.296	109.5
	12+12	3.232	68.5	1.484	125.3
[2]	12+12+12	2.365	50.1	1.294	109.3
[2]	12+12+12+12	1.717	36.4	0.879	74.3
	Seconds				
2483	0	4.792	.....	1.152	.....
	30	4.589	95.8	1.412	122.6
	30+30	3.360	70.1	1.305	113.3
[2]	30+30+30	2.523	52.7	1.287	111.7
[2]	30+30+30+30	2.149	44.8	1.046	90.8
2652	0	5.178	.....	1.145	.....
	40	5.009	96.7	1.559	136.2
	40+40	4.230	81.7	1.614	141.0
[2]	40+40+40	3.270	62.9	1.418	124.0
[2]	40+40+40+40	2.510	48.5	1.186	103.6
2967	0	5.094	.....	1.346	.....
	200	5.062	99.4	1.501	111.5
	200+200	4.936	96.9	1.549	115.1

[1] The mean is of 500 cells in each case from representative cultures.
[2] Many disintegrated cells are present.

The shorter the wave length, the greater is the effect on the algal cells; for example, the cells stimulated by four exposures to 2352 A. are 36.4 percent of the length of the cells of the control, whereas those exposed four times to 2652 A. are 48.5 percent of the length of the cells of the control. The difference in decrease in size of the cells exposed to separate wave lengths indicates that each wave length has a specific effect upon the cells.

Plate 1 shows photomicrographs of representative cells from the five sets of cultures under 2352 A. The difference in size of the cells of the cultures exposed to different stimulative amounts of the ultraviolet is evident.

DISCUSSION

It will be seen by the following tabulation that the exposures to radiation were adjusted so that the stimulation of multiplication was nearly in the same proportion for all four wave lengths on first stimulation. The mean factors of multiplication in tables 1, 2, 3, and 4, control being unity, are as follows for successive equal stimulations.

Wave length, A.	2352	2483	2652	2967
Factor on 1st stimulation	1.54	1.65	1.75	1.62
Factor on 2nd stimulation	3.30	2.12	2.17	1.10
Factor on 3rd stimulation	3.52	2.88	2.75	...
Factor on 4th stimulation	4.70	3.90	4.65	...

With the exception of the fourth stimulation for wave length 2652 A. these numbers follow approximately the general law that the longer the wave length the less effective for multiplication are successive equal stimulations.

It is very interesting to compare the results with the measurements of length and width after successive stimulations. The mean factors of length and width, referred to controls as unity, are given below. The column headed "volume" is found by multiplying the lengths by the squares of the width factors.

	2352			2483		
Wave length, A.	Length	Width	Volume	Length	Width	Volume
Factor 1st stimulation	.917	1.095	1.099	.958	1.226	1.440
Factor 2nd stimulation	.685	1.253	1.075	.701	1.133	.900
Factor 3rd stimulation	.501	1.093	.599	.527	1.117	.658
Factor 4th stimulation	.364	.742	.201	.448	.908	.369

	2652			2967		
Wave length, A.	Length	Width	Volume	Length	Width	Volume
Factor 1st stimulation	.967	1.362	1.794	.994	1.115	1.236
Factor 2nd stimulation	.817	1.410	1.624	.969	1.151	1.284
Factor 3rd stimulation	.629	1.238	.964	...	...	...
Factor 4th stimulation	.485	1.036	.520	...	...	...

Here we find the product of length by width, by width, which may be regarded as a rough measure of volume, continually increasing with wave length for the stimulations except in the case of the second stimulation for 2483 A. On first stimulations the volumes, according to this rough index, are greater than those of the controls for the four wave lengths, and this is the case for the two longer wave lengths also on second stimulation. But with rapid multiplication, such as occurs at the shorter wave lengths, the volumes soon fall below the controls.

The question naturally arises whether these results are caused by selection or by fundamental modification of the organisms. In short, are these permanently changed algae to be regarded as merely selected types, or as new species? If we had only the data on multiplication to guide us, we might be inclined to the hypothesis that irradiation tended to push to the fore the stronger, hardier individuals, and to destroy the weaker ones, thus raising the ratio of multiplying, and successive stimulations were merely a repetition of this process. But with the measurements of length and width in view, we see that the greater the stimulated ratio of multiplication, the smaller and presumably weaker the individuals become, and the more and more altered their shape as measured by ratio of longitudinal and transverse axes. Since they become so much smaller, but at the same time so much more rapidly multiplying, when irradiated with the shorter, most effective, wave lengths, does it not seem to point to a deep-seated change in the organism, rather than a mere selection?

II. THE DECREASED SENSITIVITY OF STIMULATED CELLS TO LETHAL EXPOSURES OF THE ULTRAVIOLET

The research described below was undertaken with the purpose of comparing the lethal sensitivity to the ultraviolet of the descendants of the treated cells with the descendants of the untreated cells.

EXPERIMENTAL PROCEDURE

The cultures of algae had been growing since the time of irradiation each in 100 cc. of Detmer 1/3 solution in a 300-cc. Erlenmeyer flask. Each culture was poured over the surface of Detmer 1/3, 2 percent agar which had gelled over a ground glass plate (8 x 10 cm.) resting on the bottom of a large petri dish (15 cm. in diameter). The petri dishes, plates, and Detmer 1/3 agar had been sterilized previously at 20 pounds pressure for 20 minutes in the autoclave. The petri dishes were covered immediately after the cultures had been added and were then placed on a table under four fluorescent daylight lamps, each of which was 30 watts and 89.5 cm. in length, so installed as to give an intensity of 300 foot-candles on the plates of algae. The cultures were illuminated for 12 hours of each 24 hours.

After 3 or 4 days, when the algae had started to grow on the agar, the excess liquid was decanted. The petri dish cultures were then allowed to grow for about a month, when the surfaces were completely and uniformly covered with green algal growth.

The agar plate covered with green cells was then cut out of the surrounding agar in the petri dish and placed upright in a closed sterile container with a quartz window. A decker was arranged in front of the slit of the crystal quartz prism spectrograph to permit the exposure of different portions of the plate for different lengths of time. The intensities of the wave lengths are presented in table 8. When the plate was removed from the spectrograph after irradiation, it was placed in a sterile covered petri dish, where it remained for observation under natural conditions of day and night at a temperature of about 22° C.

Untreated cultures that had been inoculated at the time of the inoculation of the treated algae were poured on the agar plates and irradiated at the same time and in the same manner as the treated algae.

The cultures were observed daily after irradiation and each decolorized region corresponding to a lethal wave length was recorded as soon as it appeared.

RESULTS

The algal cultures that had been previously stimulated with 2967 A. behaved very much like their corresponding controls. The decolorized regions appeared on both sets of plates at approximately the same time.

However, the algal cultures that had been previously stimulated with 2352, 2483, or 2652 A. behaved differently from their controls. All the decolorized regions appeared more quickly on the plates of controls than on the corresponding plates of previously stimulated algae.

Tables 6, 7, and 8 show the means of days of appearance of the decolorized regions after exposure to the full short wave length

TABLE 6.—*Exposure of stimulated cultures to the ultraviolet spectrum for 5 minutes*

Radiotoxic regions[1]	Appearance of radiotoxic regions in days after irradiation of cultures previously stimulated by monochromatic ultraviolet as follows:								
	2352 A. (3)[2]	2483 A. (2)	2652 A. (3)	Average of means	Control cultures			Average of means	Ratio of means $\frac{S}{C}$
					2352 A. Group (4)	2483 A. Group (2)	2652 A. Group (2)		
A.	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	
2536	5.3	5.0	5.0	5.1	3.5	2.0	2.0	2.5	2.0
2652	4.3	4.0	5.3	4.5	3.0	2.0	2.0	2.3	2.0
2804	6.0	5.5	6.0	4.8	3.0	4.0	2.0	3.0	1.6

[1] See table 8 for intensities of radiotoxic regions.
[2] The figures in parentheses indicate the number of cultures of which the means are given.

TABLE 7.—*Exposure of stimulated cultures to the ultraviolet spectrum for 25 minutes*

Radiotoxic regions[1]	Appearance of radiotoxic regions in days after irradiation of cultures previously stimulated by monochromatic ultraviolet as follows:								
	2352 A. (3)[2]	2483 A. (3)	2652 A. (3)	Average of means	Control cultures: 2352 A. Group (4)	Control cultures: 2483 A. Group (2)	Control cultures: 2652 A. Group (2)	Average of means	Ratio of means $\frac{S}{C}$
A.	*Days*	*Days*	*Days*		*Days*	*Days*	*Days*		
2483	6.0	9.3	6.3	7.2	4.7	2.7	2.5	3.3	2.2
2536	5.3	3.7	5.3	4.8	7.0[3]	2.0	2.0	2.0	2.4
2576	10.0	13.0	7.0	10.0	9.0[3]	5.0	4.5	4.8	2.1
2602	20.0	9.0	8.5	12.5	3.0	7.0	6.5	5.5	2.3
2652	4.3	3.7	4.3	4.1	3.7	2.0	2.0	2.6	1.6
2699	6.3	6.7	7.0	6.7	5.5	2.7	5.0	4.4	1.5
2753	8.0	9.7	7.0	8.2	3.8	4.7	5.0	4.5	1.8
2804	4.3	4.0	4.3	4.2	6.0	2.0	2.0	3.3	1.3
2894	9.0	7.0	5.5	7.2	.....	6.0	3.0	4.5	1.6
2925	.....	6.0	9.0	7.5	.....	6.0	5.0	5.5	1.4

[1] See table 8 for intensities of radiotoxic regions.
[2] The figures in parentheses indicate the number of cultures of which the means are given.
[3] Wild.

TABLE 8.—*Exposure of stimulated cultures to the ultraviolet spectrum for 60 minutes*

Radiotoxic regions	Ergs/sec./cm.[2]	Appearance of radiotoxic regions in days after irradiation of cultures previously stimulated by monochromatic ultraviolet as follows:								
		2352 A. (3)[1]	2483 A. (2)	2652 A. (3)	Average of means	Control cultures: 2352 A. Group (3)	Control cultures: 2483A. Group (2)	Control cultures: 2652 A. Group (2)	Average of means	Ratio of means $\frac{S}{C}$
A.		*Days*	*Days*	*Days*		*Days*	*Days*	*Days*		
2250	214	.....	6.0	16.0	11.0	6.0	6.0	3.0	5.0	2.2
2300	459	11.0	6.0	12.5	9.8	5.0	5.0	4.0	4.7	2.1
2323	235	.....	6.0	18.0	12.0	5.6	9.0	2.0	5.5	2.2
2352	641	9.5	6.0	12.5	9.3	4.7	7.0	1.5	4.4	2.1
2378	1004	10.0	6.0	5.0	7.0	4.5	6.0	1.0	3.8	1.8
2399	1068	.....	6.0	5.7	5.9	4.0	2.0	1.5	2.5	2.4
2447	342	13.0	7.0	.....	10.0	6.0	.....	4.5	5.3	1.9
2463	444	12.0	6.0	.....	9.0	5.0	.....	4.0	4.5	2.0
2483	2500	6.0	3.0	4.0	4.3	3.0	2.0	2.0	2.7	1.6
2536	7265	5.0	3.0	3.3	3.8	1.6	2.0	1.0	1.5	2.5
2576	572	10.0	5.5	7.7	7.7	4.6	5.5	4.5	4.8	1.6
2602	286	11.0	7.0	7.0	8.3	5.0	5.5	5.0	5.2	1.6
2652	6303	4.3	3.0	3.3	3.5	1.6	1.5	1.0	1.4	2.5
2699	1400	5.0	4.0	7.0	5.3	2.5	2.0	3.5	2.7	2.0
2753	1026	6.3	4.0	6.5	5.6	3.8	2.0	3.5	3.1	1.8
2804	3739	4.3	3.0	3.3	3.5	1.6	1.5	1.5	1.5	2.3
2894	1603	8.0	5.0	5.0	6.0	6.0	3.5	2.5	4.0	1.5
2925	572	12.0	6.5	6.0	8.2	6.8	3.5	4.5	4.9	1.7

[1] The figures in parentheses indicate the number of cultures of which the means are given.

ultraviolet spectrum ranging from wave lengths 2250 A. to 3130 A. on the three sets of stimulated and control plates of algae. As there were three different exposures on each plate, namely, 5 minutes, 25

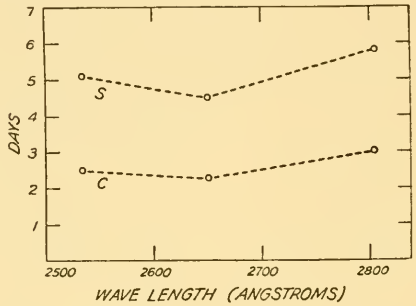

FIG. 1.—Averages of means of days of appearance of lethal regions in cultures of algae exposed for 5 minutes to the ultraviolet spectrum (based on data from table 6). S=stimulated cultures, C=control cultures.

FIG. 2.—Averages of means of days of appearance of lethal regions in cultures of algae exposed for 25 minutes to the ultraviolet spectrum (based on data from table 7). S=stimulated cultures, C=control cultures.

minutes, and 60 minutes, the results are tabulated in three separate tables according to the exposure time. Figures 1, 2, and 3 show the graphs made from the averages of the means given in tables 6, 7,

and 8. The reader's attention is drawn to the general similarity of the curve shown in figure 3 with that of figure 2 in Meier, 1936.

Plate 2 shows spectrograms of two algal plates of untreated algae and algae that had been stimulated with 2483 A. for 30 seconds. Both plates were prepared at the same time and exposed to the short wave lengths of the ultraviolet spectrum ranging from 2250 to 3130 A. for (1) 25 minutes, (2) 5 minutes, and (3) 60 minutes. Notice how much more clearly the lethal regions appear in the untreated plate than on the treated plate and also the greater number of lethal lines

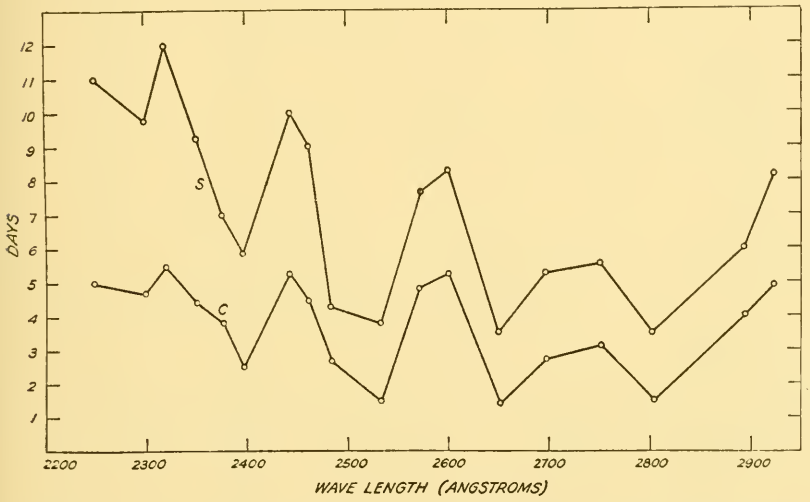

FIG. 3.—Averages of means of days of appearance of lethal regions in cultures exposed for 60 minutes to the ultraviolet spectrum (based on data from table 8). S = stimulated cultures, C = control cultures.

on the untreated plate than on the treated plate, thus demonstrating the greater sensitivity of the untreated cells to the lethal regions of the ultraviolet.

The above results relate to algae that have been stimulated by one single irradiation.

More plates were made of algae that had been stimulated by 2, 3, and 4 successive exposures to 2652 A. Unfortunately, those plates which had been stimulated twice became infected with *Chlorella pyrenoidosa,* so that it was necessary to discard them. However, the means of days of appearance of lethal regions in cultures stimulated by three exposures and four exposures to 2652 A. have been sum-

marized and tabulated together with the means of the controls and single stimulative exposures to 2652 A. (from tables 6, 7, and 8) in tables 9, 10, and 11.

TABLE 9.—*Exposure of stimulated cultures to the ultraviolet spectrum for 5 minutes*

Radiotoxic regions[1]	Appearance of radiotoxic regions in means of days after irradiation of cultures stimulated by successive exposures to 2652 A. as follows:			
	Control	Exposure 40 sec.	Exposures 40+40+40 sec.	Exposures 40+40+40+40 sec.
A.	*Days*	*Days*	*Days*	*Days*
2536	2.0	5.0	6.8	6.5
2652	2.0	5.3	5.8	6.5
2804	2.0	6.0	8.0	6.5

[1] See table 8 for intensities of radiotoxic regions.

TABLE 10.—*Exposure of stimulated cultures to the ultraviolet spectrum for 25 minutes*

Radiotoxic regions[1]	Appearance of radiotoxic regions in means of days after irradiation of cultures stimulated by successive exposures to 2652 A. as follows:			
	Control	Exposure 40 sec.	Exposures 40+40+40 sec.	Exposures 40+40+40+40 sec.
A.	*Days*	*Days*	*Days*	*Days*
2483	2.5	6.3	7.0	6.5
2536	2.0	5.3	5.3	6.5
2576	4.5	7.0	21.6	14.0
2602	6.5	8.5	22.6	18.0
2652	2.0	4.3	4.8	6.5
2699	5.0	7.0	10.0	6.5
2753	5.0	7.0	17.6	8.5
2804	2.0	4.3	6.0	6.5
2894	3.0	5.5	63.0	17.5
2925	5.0	9.0	.....	.....

[1] See table 8 for intensities of radiotoxic regions.

RESULTS

In each case, the stimulated cells are less sensitive to the lethal ultraviolet than the control cells. The fact that the sensitivity did not increase in proportion with the stimulation is probably due to the number of disintegrated cells that were found in the cultures that had been irradiated three or four times.

TABLE 11.—*Exposure of stimulated cultures to the ultraviolet spectrum for 60 minutes*

Radiotoxic regions[1]	Appearance of radiotoxic regions in means of days after irradiation of cultures stimulated by successive exposures to 2652 A. as follows:			
	Control	Exposure 40 sec.	Exposures 40+40+40 sec.	Exposures 40+40+40+40 sec.
A.	*Days*	*Days*	*Days*	*Days*
2250	3.0	16.0	8.7	10.0
2300	4.0	12.5	6.0	6.5
2323	2.0	18.0	5.8	6.5
2352	1.5	12.5	4.5	6.5
2378	1.0	5.0	4.0	6.5
2399	1.5	5.7	3.8	6.5
2447	4.5	.....	.....	.....
2463	4.0	.....	.....	.....
2483	2.0	4.0	3.5	6.5
2536	1.0	3.3	3.4	6.5
2576	4.5	7.7	5.5	12.5
2602	5.0	7.0	40.8	13.5
2652	1.0	3.3	3.5	6.5
2699	3.5	7.0	4.5	6.5
2753	3.5	6.5	4.5	6.5
2804	1.5	3.3	3.5	6.5
2894	2.5	5.0	4.0	6.5
2925	4.5	6.0	7.5	8.5

[1] See table 8 for intensities of radiotoxic regions.

CONCLUSIONS

Cells of the alga *Stichococcus bacillaris* were exposed repeatedly to stimulative amounts of four short wave lengths of the ultraviolet. After four successive irradiations, the cells exposed to 2352 A. were stimulated to a growth rate (as measured by number of cells) of 4.7 times the control cells; those cells exposed to 2483 A. to 3.9 times the control cells; and those exposed to 2652 A. to 4.65 times the control cells. The cells were stimulated by the first irradiation with 2967 A. to 1.62 times the control cells, but they did not respond to the second irradiation and their rate of increase was practically the same as that of the control cells.

The stimulation of multiplication was approximately in the same proportion for all four wave lengths on first stimulation. The numbers for successive equal stimulations by each wave length followed approximately the general law that the longer the wave length the less effective for multiplication are successive equal stimulations.

The length of the cells decreased with each stimulative exposure; the width was slightly greater than that of the control cells except in the fourth stimulations of 2352 A. and 2483 A. The greater the stimulated ratio of multiplication, the smaller and weaker the indi-

viduals became and the more altered their shape as measured by ratio of longitudinal and transverse axes.

In general, the volumes continually increase with wave length for the stimulations. On first stimulations, the volumes are greater than those of the controls for the four wave lengths and on second stimulations for the two longer wave lengths. With rapid multiplication as occurs at the shorter wave lengths, the volumes soon fall below those of the controls.

The cells stimulated by monochromatic wave lengths of the ultraviolet are less sensitive to lethal exposures of the ultraviolet spectrum than are the control cells.

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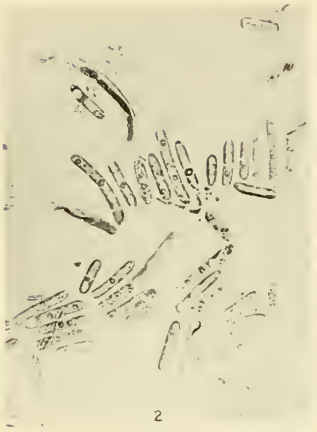
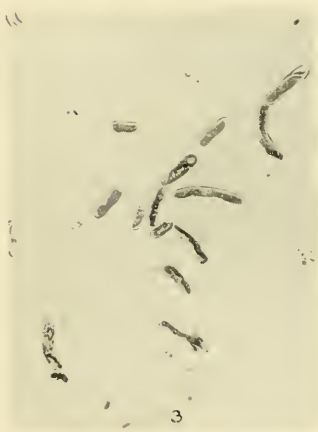
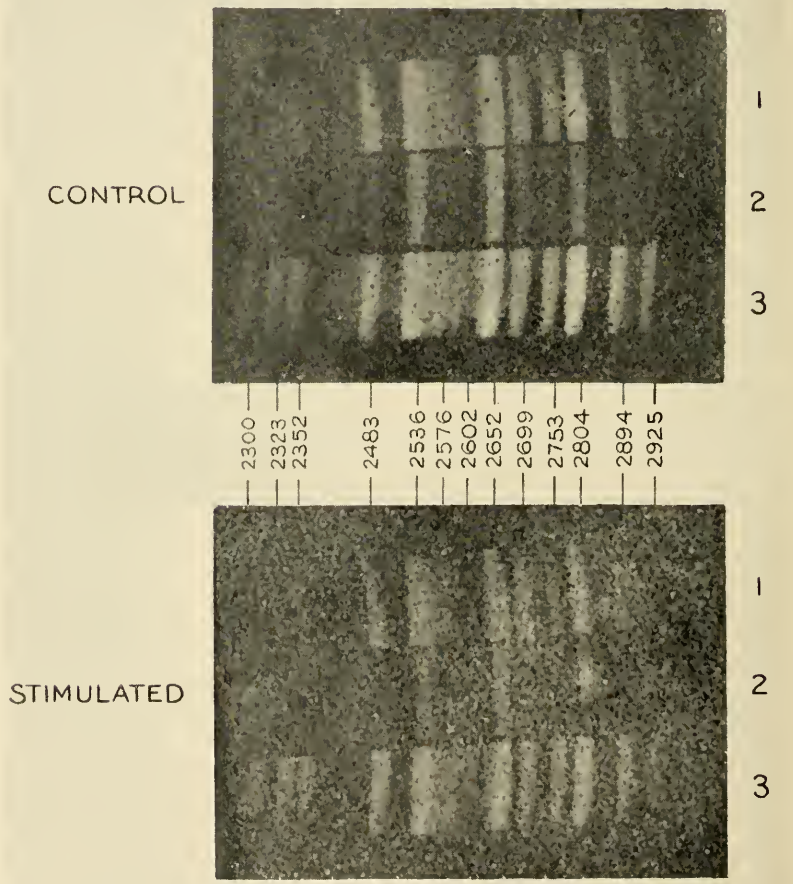




Difference in size of algal cells in cultures exposed to varying stimulative amounts of 2352 A.: 1, Control; 2, exposed for 12 minutes; 3, exposed for 12 + 12 minutes; 4, exposed for 12 + 12 + 12 minutes; 5, exposed for 12 + 12 + 12 + 12 minutes; 6, disintegrating cells exposed to 2652 A. for 40 + 40 + 40 + 40 seconds. × 250.



Comparative spectrograms of untreated algae and stimulated algae exposed to short wave lengths of the ultraviolet spectrum for (1) 25 minutes, (2) 5 minutes, and (3) 60 minutes.