SMITHSONIAN MISCELLANEOUS COLLECTIONS
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(With Four Plates)

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INTRODUCTION

During the course of previous quantitative research on the lethal effect of 21 wave lengths of the ultraviolet ranging from 2250 to 3130 A. on a green alga, the author (Meier, 1932, 1934, 1936) observed from time to time accelerated increase in cell mass with slightly less exposure than the lethal exposure that destroyed the plant cells. The purpose of the present study is to show quantitatively the stimulative action of definite short wave lengths of the ultraviolet on growth as measured by cell multiplication in a unicellular green alga, a stimulative action that has persisted over a period of 2 years in the same cultures without additional treatment.

By means of a quartz mercury-vapor arc and a fused-quartz prism spectrograph, exposures were made to the wave lengths 2652 and 2967 A. A spectrograph with crystal quartz prisms was used 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 spectroscopic manipulations and physical measurements were made by Dr. E. D. McAlister, of the Division of Radiation and Organisms.

I wish to express my appreciation to Dr. C. G. Abbot, Secretary of the Smithsonian Institution, for his counsel and assistance during the progress of this investigation. I am also grateful to Dr. E. S. Johnston and other members of the Division of Radiation and Organisms who have aided me in this research.

LITERATURE REVIEW

Several investigators have studied the problem of stimulation of growth resulting from irradiation by short wave lengths of the ultraviolet which are generally considered lethal. The chief difficulty consists in the correct control of conditions for a quantitative determination of the stimulating exposure for an organism.

Gilles (1938), in his treatise of 286 pages on "Effets des Rayons Ultra-violets sur les Végétaux Supérieurs," offers a very comprehensive survey of all the research that has been carried on up to 1938 on the destructive and favorable effects of the ultraviolet on both higher plants and microorganisms. This book, supplemented by the review made by Wynd and Reynolds (1935) of all the investigations that have been made on ultraviolet and respiratory phenomena, and by the review made by Sperti, Loofbourow, and Dwyer (1937) on the stimulative effect of ultraviolet on microorganisms, covers the literature thoroughly.

In this paper I shall mention briefly the principal investigations that have been made on the stimulative effect of short wave lengths of the ultraviolet on microorganisms.

Chavarria and Clark (1924) found that when certain pathogenic fungi very resistant to ultraviolet were given sublethal exposures of ultraviolet from a quartz mercury arc, they showed marked evidence of stimulation and grew larger than the controls. They observed that the stage of stimulation was brief and that lethal action followed rapidly in nonpigmented cells, but where pigment formed during irradiation, the cell was protected and its whole reaction was slowed down so that the stage of stimulation was relatively prominent and the lethal action was delayed. Fungi that are less resistant to the ultraviolet when treated with sublethal exposures produced feeble colonies, which in a few days grew to the size of the controls and when autoinoculation of the culture medium occurred, the daughter colonies were unusually vigorous.

Porter and Bockstahler (1928) report stimulated spore formation in the fungus *Colletotrichum* when exposed to radiations between 3630 and 3650 A. produced by a Cooper-Hewitt mercury arc.

Stevens (1928), in his researches on perithecia formation in fungi, observed that a "very common, though not universal, effect of irradiation is to increase sexual or asexual production if such occurs normally and in some species to induce the sexual stage where it does not occur normally." The exposure was to full radiation from a Cooper-Hewitt mercury arc. Half of the colony on the agar plate was shaded

with cardboard to serve as a control. By means of various screens the activating region was found to lie probably between 2760 and 3130 A.

Nadson and Philippov (1928) used a quartz mercury-vapor lamp to irradiate petri-dish cultures of pure cultures of yeasts and fungi covered with thick glass or metal with an opening in the center. The growth directly under the opening was completely destroyed by the ultraviolet. The part protected by the screen developed normally, but immediately surrounding the sterile zone was a bracelet of very intense growth where the culture partially protected by the screen had been touched by only the oblique rays. The yeasts in this region presented exaggerated budding; for example, the cell of the yeast *Nadsonia fulvescens,* which normally forms from one to three buds, in this case formed at its extremities whole groups or bouquets of buds. The mother cell, excited by the irradiation to such intense multiplication, swelled up, then, worn out, degenerated and perished. The group of young cells, dissociated by this fact, continued to live independently and to multiply by budding. In the zone of stimulation, *Mucor guilliermondii* asexually produced large quantities of sporangia, while on the other hand, sexual reproduction of *Mucor genevensis* was stimulated.

Ramsey and Bailey (1930) found a definite stimulation of spore production in cultures of *Macrosporium tomato* and *Fusarium cepae* on exposure to ultraviolet radiation from a quartz mercury arc. The greatest amount of spore production occurred when filters with the lower limits of transmission between 2535 and 2800 A. were used. There was stimulation also with irradiations of 2535 A. and shorter wave lengths, but with these exposures there was also some lethal effect and inhibition of mycelial development.

Hutchinson and Ashton (1930), using a quartz mercury-vapor lamp and a Hilger monochromatic illuminator, found that irradiation with the short wave length 2700 A. caused early retardation of growth followed by stimulation in the fungus *Colletotrichum phomoides* and in yeast. Early sporulation of *Colletotrichum* resulted from irradiation with 3132, 3022, 2804, 2700, 2054, and 1854 A.; early acervuli development occurred after irradiation with 2535 A.

Smith (1935) reports stimulated spore production in *Fusarium eumartii* Carp., regardless of the growth rate of the fungus after irradiation with a Cooper-Hewitt mercury arc lamp.

De Fazi and de Fazi (1915) describe experiments showing that *Saccharomyces opuntiae,* when irradiated with ultraviolet, lived, reproduced, and fermented more actively.

Lindner (1922) also irradiated yeast with ultraviolet and found that it increased the speed of fermentation. He reports that 20 to 30 percent of the cells in the irradiated culture died, thus indicating that he had given the cells a sublethal dose of ultraviolet.

Owen (1933) reports increased fermentative power of yeast caused by irradiation with a carbon arc, which is rich in wave lengths 2300 to 3100 A.

Hollaender and Curtis (1935), on irradiating colonies of bacteria, *Escherichia coli,* with monochromatic ultraviolet radiation below 3000 A. with exposures that killed some of the organisms but not all of them, found that growth of the surviving irradiated bacteria was retarded as compared with the control (nonirradiated) bacteria. But when the bacteria had completed their growth, the same number of organisms was present in both the irradiated and the control suspensions. The irradiated culture increased in number quite rapidly in the early part of the "lag phase," then slowed down, thus suggesting stimulation. Tests showed that the increased growth of the irradiated bacteria was not produced by the decomposition products of the dead bacteria or by the excretion of any substance by the irradiated organisms. The authors thought that stimulation was suggested, but the possibility of recovery of the irradiated bacteria should not be entirely excluded.

Hollaender and Duggar (1937), in their treatment of yeast and bacteria with sublethal doses of 2650 A., observed that the survivors of the irradiation proliferated when transferred to salt solutions in which the controls died.

Loofbourow and others (1938, 1939), from their intensive studies on the stimulating effect of ultraviolet and other lethal agents in sublethal quantities on cells, believe that the effect is due to the production by injured living cells of "wound hormones," released as a physiologic response to injury into the intercellular medium. Their work is on embryo chicks and mouse tissues, yeast, and bacteria.

EXPERIMENTAL PROCEDURE

In the experiments on the lethal effect of the ultraviolet rays, the alga *Chlorella vulgaris* was found to be well adapted to the research because of the uniformity and speed with which the single oval cells covered the surface of the agar plates used. For experimentation involving direct counting, this alga is highly unsatisfactory owing to the minute and irregular size of the cells, which vary from 3 to 5 μ, and to the fact that the cell multiplies by oval or elliptical spores, which may range in number from two to four.

The unicellular green alga *Stichococcus bacillaris* Naegeli lends itself more 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 2 to 2.5 μ in width and 4 to 8 μ 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. (See pl. 3.) Filaments of more than two cells were rarely observed in my 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 sulphate	0.25	"
Potassium acid phosphate	0.25	"
Ferric chloride	0.002	"
Distilled water	1.0	liter

Before irradiation, algae 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 wire stirrer inserted through the cork so that the culture could be stirred during irradiation. (See fig. 1.) 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 ray of ultraviolet, 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 were 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 grew equally well in the rubber-stoppered flask and the flask with a cotton

FIG. 1.—Quartz tube with flattened side and stirrer. Natural size.

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 same 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 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.

THE GROWTH CHAMBER

During the early experiments conducted in 1937 the algae in the Erlenmeyer flasks were grown during the 2 weeks following irradiation in natural conditions of day and night at variable room temperature in the north window on the eleventh floor of the Smithsonian flag tower. These environmental conditions of growth proved to be entirely too variable, as shown by the results for the experiments with algae grown under uncontrolled conditions presented in tables 1-4.

To arrange an ideal environment for the growth of the cultures after irradiation, an electric refrigerator was equipped with a thermostat, which regulated the temperature of the inner chamber at a constant temperature of 24° C. during the day and 22° C. at night. The new fluorescent daylight lamps were tested and proved to produce better growth conditions for the algae than varying daylight (see pl. 1). A set of four of these daylight lamps, each of which was 15 watts and

TABLE 1.—*Growth of the alga* Stichococcus bacillaris *Naegeli in relation to wave length 2352 A*

	Uncontrolled growth conditions						Controlled growth conditions					
Experiment No.	1		2		3		1		2		3	
Date	Apr. 28, 1937		May 18, 1937		June 3, 1937		March 6, 1939		March 15, 1939		March 28, 1939	
Intensity, ergs/sec.cm.^{2}	600		590		590		600		570		580	
Exposure	Growth rate	÷3	Growth rate	÷1.7	Growth rate	÷1.7	Growth rate	÷1.4	Growth rate	÷1.4	Growth rate	÷1.4
Minutes												
0 (control)	3.0	1	2.6	1	1.76	1	1.4	1	1.4	1	1.4	1
	3.5	1	1.7	1	1.00	1	1.4	1	1.5	1	1.8	1
											1.4	1
											1.4	1
2	2.8	0.93										
	3.8	1.3										
4	3.4	1.1					1.9	1.4	1.3	0.92		
	3.6	1.2					2.2	1.6	1.5	1.0		
6	3.1	1.0										
	2.8	0.93										
8	3.6	1.2	1.1	0.64	2.02	1.2	2.4	1.7	1.1	0.79	1.6	1.1
	4.7	1.6	1.2	0.71	2.60	1.5	2.2	1.6	1.1	0.79	1.3	0.93
											1.5	1.1
											1.6	1.1
12	5.1	1.7	4.5	2.6	4.48	2.6	2.5	1.8	1.4	1.0	2.2	1.6
	4.1	1.4	4.8	2.8	4.54	2.7	3.2	2.3	1.1	0.79	2.0	1.4
											2.1	1.5
											2.0	1.4
16			3.5	2.1	1.05	0.62	1.1	0.79	2.0	1.4		
			2.0	1.2	1.86	1.1	1.1	0.79	1.3	0.93		
20			1.3	0.76	0.50	0.29	1.0	0.71	0.50	0.36		
			1.7	1.0	0.52	0.31	1.1	0.79	0.76	0.54		
24			1.6	0.94								
			1.5	0.88								
30					0.70	0.41						
					0.57	0.34						

TABLE 2.—*Growth of the alga* Stichococcus bacillaris *Naegeli in relation to wave length 2483 A*

	Uncontrolled growth conditions		Controlled growth conditions					
Experiment No.	1		1		2		3	
Date	Nov. 15, 1937		April 11, 1939		April 19, 1939		April 27, 1939	
Intensity, ergs/sec.cm.^{2}	2660		2620		2650		2640	
Exposure	Growth rate	÷1.5	Growth rate	÷1.4	Growth rate	÷1.6	Growth rate	÷1.3
Seconds								
0 (control)	.....	.....	1.4	1	1.5	1	1.3	1
	1.5	1	1.4	1	1.7	1	1.2	1
10	.....	.....	.....	.....	.....	.....	1.5	1.2
	.....	.....	.....	.....	.....	.....	1.5	1.2
15	5.0	3.3	.....	.....	.....	.....	.....	.....
	1.9	1.3	.....	.....	.....	.....	.....	.....
20	.....	.....	.....	.....	.....	.....	1.9	1.6
	.....	.....	.....	.....	.....	.....	1.9	1.6
30	3.5	2.3	7.9	5.6	2.3	1.4	.....	.....
	2.6	1.7	8.5	6.0	2.4	1.5	.....	.....
40	.....	.....	.....	.....	.....	.....	1.3	1
	.....	.....	.....	.....	.....	.....	1.3	1
60	2.3	1.5	1.9	1.4	1.5	1	.....	.....
	3.3	2.2	2.0	1.4	1.6	1	.....	.....
80	.....	.....	.....	.....	.....	.....	1.2	1
	.....	.....	.....	.....	.....	.....	1.4	1
90	2.4	1.6	.....	.....	.....	.....	.....	.....
	2.7	1.8	.....	.....	.....	.....	.....	.....
100	.....	.....	1.0	0.71	1.2	0.75	.....	.....
	.....	.....	1.0	0.71	1.2	0.75	.....	.....
120	1.1	0.7	.....	.....	.....	.....	.....	.....
	0.65	0.4	.....	.....	.....	.....	.....	.....
180	.....	.....	1.2	0.86	1.1	0.69	.....	.....
	.....	.....	1.5	1.1	1.2	0.75	.....	.....

TABLE 3.—*Growth of the alga* Stichococcus bacillaris *Naegeli in relation to wave length 2652 A*

	Uncontrolled growth conditions				Controlled growth conditions					
Experiment No.	1		2		1		2		3	
Date	March 1, 1937		March 31, 1937		Dec. 8, 1938		Dec. 21, 1938		Jan. 11, 1939	
Intensity, ergs/sec.cm.^{2}	2100		2100		1950		1980		1950	
Exposure	Growth rate	÷0.89	Growth rate	÷1.6	Growth rate	÷1.7	Growth rate	÷1.7	Growth rate	÷1.6
Seconds										
0 (control)	0.86	1	2.6	1	1.8	1	1.7	1	1.5	1
	0.91	1	1.5	1	1.6	1	1.6	1	1.6	1
									1.4	1
									1.7	1
10	1.4	1.6	2.2	1.3	1.9	1.1	2.7	1.7		
	2.1	2.3	1.5	0.96	1.7	1.0	2.8	1.8		
20	2.0	2.3	4.6	2.9	1.9	1.1	2.5	1.6	1.5	0.93
	1.6	1.8	4.5	2.8	1.8	1.1	2.6	1.6	2.0	1.3
									1.8	1.1
									1.9	1.2
30	3.2	3.5	4.6	2.9	1.5	0.82	2.6	1.6		
	2.3	2.6			1.2	0.71	2.6	1.6		
40	4.2	4.7	6.8	4.3	3.9	2.2	1.7	1.1	3.0	1.9
	4.2	4.7	7.1	4.4	3.3	1.9	2.9	1.9	2.0	1.3
									2.6	1.6
									2.4	1.5
60	0.88	0.99	1.0	0.63	0.71	0.41	1.1	0.69		
	0.87	0.98	1.0	0.63	0.79	0.46	0.92	0.58		

TABLE 4.—*Growth of the alga* Stichococcus bacillaris *Naegeli in relation to wave length 2967 A*

	Uncontrolled growth conditions				Controlled growth conditions					
Experiment No.	1		2		1		2		3	
Date	July 19, 1937		Sept. 24, 1937		Feb. 1, 1939		Feb. 6, 1939		Feb. 27, 1939	
Intensity, ergs/sec.cm.^{2}	2500		2380		2400		2350		2370	
Exposure	Growth rate	÷9	Growth rate	÷2.5	Growth rate	÷1.4	Growth rate	÷2.2	Growth rate	÷1.4
Seconds										
0 (control)	7.5	1	2.3	1	1.4	1	2.1	1	1.4	1
	10.6	1	2.6	1	1.4	1	2.2	1	1.6	1
	.....	.....	.....	.....	.....	.....	.....	.....	1.3	1
	.....	.....	.....	.....	.....	.....	.....	.....	1.4	1
33	6.3	0.69	1.8	0.72	.....	.....	.....	.....	.....	.....
	5.0	0.56	1.6	0.62	.....	.....	.....	.....	.....	.....
67	5.7	0.63	1.5	0.75	1.4	1	1.8	0.80	.....	.....
	11.0	1.2	1.9	0.60	1.4	1	1.9	0.86	.....	.....
100	9.4	1.0	3.5	1.4	1.2	0.87	1.5	0.69	.....	.....
	7.2	0.80	3.2	1.3	1.3	0.95	1.3	0.59	.....	.....
133	9.5	1.1	7.2	2.9	1.2	0.85	1.8	0.81	1.9	1.4
	12.8	1.4	7.3	2.9	1.4	1.0	1.7	0.77	1.6	1.1
	.....	.....	.....	.....	.....	.....	.....	.....	1.9	1.4
	.....	.....	.....	.....	.....	.....	.....	.....	1.8	1.3
200	2.2	0.24	4.5	1.8	2.1	1.5	2.9	1.3	2.6	1.9
	2.2	0.24	5.0	2.0	2.4	1.7	2.4	1.1	2.7	1.9
	.....	.....	.....	.....	.....	.....	.....	.....	2.7	1.9
	.....	.....	.....	.....	.....	.....	.....	.....	2.6	1.9
260	.....	.....	.....	.....	0.51	0.36	1.1	0.50	.....	.....
	.....	.....	.....	.....	0.83	0.59	1.4	0.64	.....	.....

18 inches in length, 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 (see pl. 2) during their growth period of two weeks.

RESULTS

All the results have been tabulated in tables 1-10 for convenient reference.

THE STIMULATED GROWTH RATE

The results of the cell counts are tabulated in tables 1-4 and 6-9. In each case, the growth rate represents the final count made 2 weeks after irradiation divided by the initial count made directly after irradiation. Each growth rate of an irradiated culture was then divided by the growth rate of the control to obtain the final growth ratio.

In plotting the curves (see fig. 2), all the points for the final growth ratios were considered. This includes the points found in the set of experiments run with old stimulated cultures as described later. The figures near the points in the curves represent the number of observations entering into the mean. In plotting the curve for 2483 A., certain observations that seemed very wild were rejected in the preferred diagram, drawn with full lines. The dotted curves represent all the observations.

As shown by studying the curves and the tables, the highest point of stimulation for all the wave lengths is at approximately two-thirds of the intensity of the lethal threshold. The greatest amount of stimulation was found by exposure to the wave length 2652 A., and the smallest amount to the wave length 2967 A. These results correspond to the radiotoxic spectral sensitivity of green algae found previously (Meier, 1936). Some stimulation was obtained by shorter exposures than the optimum to each of these four wave lengths.

To check the lethal threshold points found in the liquid cultures, the following experiment was conducted. Green algal cells growing in Detmer 1/3 were pipetted into the quartz tubes. After irradiation, the cells were pipetted into petri dishes containing sterilized Detmer 1/3 agar. They were then placed in the growth chamber and observations were made daily. The results as given below show that the lethal points correspond to those obtained in the liquid cultures.

2352 A.		2483 A.		2652 A.		2967 A.	
590 ergs/sec.cm.²		2,600 ergs/sec.cm.²		1,950 ergs/sec.cm.²		2,350 ergs/sec.cm.²	
Control	Green	Control	Green	Control	Green	Control	Green
"	"	"	"	"	"	"	"
20 min.	Lethal	1 min.	"	1 min.	Lethal	4 min.	Lethal
20 "	"	1 "	"	1 "	"	4 "	"
40 "	"	2 "	Lethal	2 "	"	6 "	"
40 "	"	2 "	"	2 "	"	6 "	"
70 "	"	3 "	"	3 "	"	9 "	"
70 "	"	3 "	"	3 "	"	9 "	"
120 "	"	5 "	"	5 "	"	12 "	"
120 "	"	5 "	"	5 "	"	12 "	"

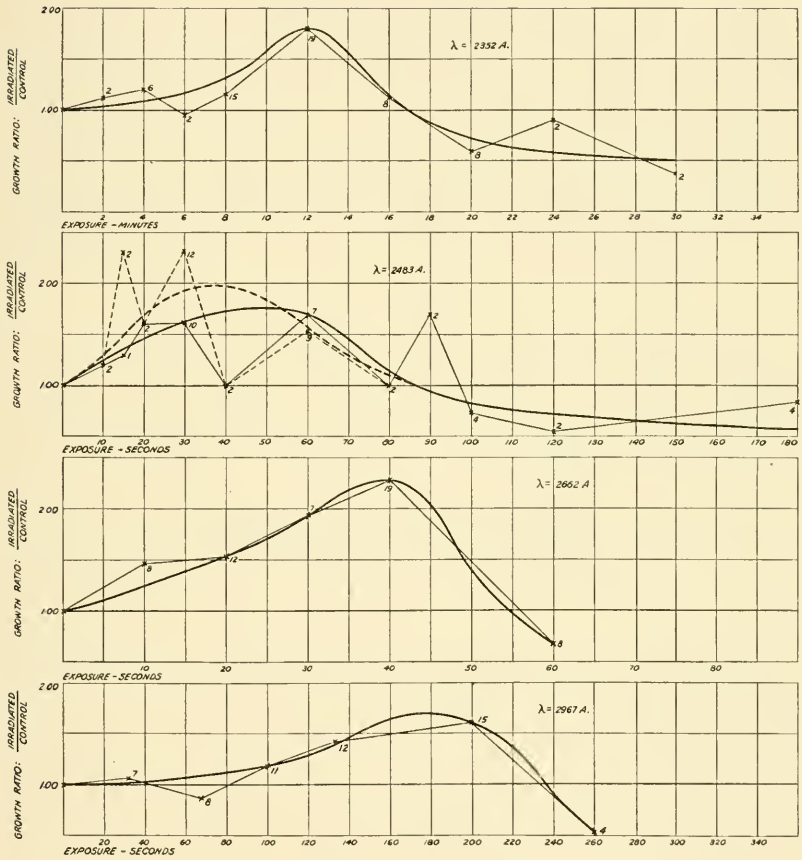

FIG. 2.—Stimulative action on cell multiplication of the alga *Stichococcus bacillaris* Naegeli resulting from exposures to ultraviolet rays. Dark line, smooth curve; light line, actual values. The figures indicate the number of observations.

STIMULATED GROWTH FROM OLD STIMULATED CULTURES

In 1939 it was observed that the old cultures that had been growing undisturbed since their growth count 2 weeks after irradiation in 1937 presented a sufficient growth of algae for weighing. Several cultures were filtered, and when the dry weights were obtained the weights of the stimulated cultures proved to be much greater than those of the controls (see table 5). The 2652 A. cultures were filtered through previously weighed filter paper, and then the precipitate and the filter paper were dried in the electric oven before weighing. The 2967 A. cultures were filtered through filter papers that had been previously dried and each weighed with its small covered aluminum pan. The filter papers of algae were then placed each in its pan in the

TABLE 5.—*Dry Weight of Algae 2 Years after Inoculation*

Wave length	Date	Exposure	Tare	Tare + Algae	Algae	$\frac{S}{C}$
A.	*1937*		*gm.*	*gm.*	*gm.*	
2652	Mar. 30	0 sec. (control)	.624	.647	.023	1
		40 sec.....	.570	.621	.051	2.2
2967	July 19	0 sec. (control) (A) .	17.993	17.998	.005	1
		200 sec. (A).....	18.187	18.208	.021	4.2
		0 sec. (control) (B) .	17.674	17.681	.007	1
		200 sec. (B).....	17.663	17.681	.018	2.6

oven and dried for a much longer period than the 2652 A. cultures. These data are of value in that they indicate continued increase in multiplication of the cells for a period of 2 years.

To check this finding, a series of experiments was performed with cultures that had been irradiated and allowed to stand undisturbed over a period of time. Flasks of fresh culture solution were inoculated with inoculum from the stimulated cultures and from the control culture of each series. Cell counts were made as described above directly after inoculation, and 2 weeks after the new series of cultures had been placed in the growth chamber. In each case, as shown in tables 6-9, the growth rate was higher in the stimulated cultures than in the control, proving that the accelerated rate of multiplication of the algal cells, increased by irradiation with the ultraviolet, had persisted for a period of 2 years.

TABLE 6.—*Persistent Stimulated Growth Obtained from Cultures Formerly Stimulated by Wave Length 2352 A*

Experiment No....	1		2		3	
Date inoculum was irradiated....	June 3, 1937		May 18, 1937		May 18, 1937	
Date of experiment	March 8, 1939		April 4, 1939		April 14, 1939	
Exposure.....	Growth rate	÷1.6	Growth rate	÷1.3	Growth rate	÷1.5
Minutes						
0 (control) ...	1.7	1	1.1	1	1.4	1
	1.8	1	1.3	1	1.3	1
	1.4	1	1.5	1	1.3	1
12.....	2.5	1.6	2.5	1.9	2.8	2.2
	2.9	1.8	2.7	2.0	3.4	2.6
	2.9	1.8	3.0	2.3	2.9	2.2

TABLE 7.—*Persistent Stimulated Growth Obtained from Cultures Formerly Stimulated by Wave Length 2483 A*

Experiment No....	1		2		3	
Date inoculum was irradiated....	Dec. 7, 1937		Dec. 7, 1937		Dec. 7, 1937	
Date of experiment	May 3, 1939		May 8, 1939		May 9, 1939	
Exposure.....	Growth rate	÷1.3	Growth rate	÷1.4	Growth rate	÷1.1
Seconds						
0 (control) ...	1.2	1	1.2	1	1.1	1
	1.2	1	1.4	1	1.1	1
	1.4	1	1.5	1	1.2	1
30.....	2.0	1.5	.....	.....	1.6	1.5
	2.6	2.0	.....	.....	1.6	1.5
	2.0	1.5	.....	.....	1.4	1.3
60.....	.....	.....	2.4	1.7	.....	.....
	.....	.....	2.2	1.6	.....	.....
	.....	.....	2.8	2.0	.....	.....

TABLE 8.—*Persistent Stimulated Growth Obtained from Cultures Formerly Stimulated by Wave Length 2652 A*

Experiment No....	1		2		3	
Date inoculum was irradiated....	Dec. 8, 1938		Jan. 11, 1939		Dec. 8, 1938	
Date of experiment	March 10, 1939		April 6, 1939		April 14, 1939	
Exposure.....	Growth rate	÷1.4	Growth rate	÷2.1	Growth rate	÷1.6
Seconds						
0 (contro') ...	1.4	1	2.2	1	1.4	1
	1.5	1	2.2	1	1.8	1
	.....	.....	2.0	1	1.7	1
	.....	.....	2.0	1	.....	.....
40.....	3.2	2.3	2.8	1.3	2.1	1.3
	3.2	2.3	2.7	1.3	2.7	1.7
	.....	.....	3.3	1.6	2.2	1.4
	.....	.....	2.6	1.3	.....	.....

TABLE 9.—*Persistent Stimulated Growth Obtained from Cultures Formerly Stimulated by Wave Length 2967 A*

Experiment No....	1		2		3	
Date inoculum was irradiated....	July 19, 1937		July 19, 1937		July 19, 1937	
Date of experiment	May 10, 1939		May 15, 1939		May 23, 1939	
Exposure.....	Growth rate	÷1.3	Growth rate	÷1.2	Growth rate	÷1.1
Seconds						
0 (control) ...	1.4	1	1.2	1	1.1	1
	1.3	1	1.2	1	1.0	1
	1.2	1	1.2	1	1.1	1
33.....	.....	.....	1.9	1.6	.....	.....
	.....	.....	1.9	1.6	.....	.....
	.....	.....	1.8	1.5	.....	.....
100.....	2.3	1.8	.....	.....	.....	.....
	2.2	1.7	.....	.....	.....	.....
	2.4	1.9	.....	.....	.....	.....
200.....	.....	.....	.....	.....	1.9	1.7
	.....	.....	.....	.....	3.1	2.8
	.....	.....	.....	.....	2.2	2.0

APPEARANCE OF THE CELLS

The lengths and the widths of 20 cells in each culture were measured with an ocular micrometer. From these data the means of the measurements of 100 cells from the most highly stimulated cultures and of 100 cells from the corresponding controls were computed and the results tabulated in table 10. The measurements of the stimulated cultures under 2352 A. are from the cultures exposed to that wave length for 12 minutes, the set under 2483 A. from those with 30 seconds' exposure, the set under 2652 A. from those with 30 and 40 seconds' exposure, and the set of measurements under 2967 A. from those with 133 and 200 seconds' exposure. The ratios were computed by dividing the mean length of the measurements for 100 cells of the stimulated cultures by the mean length for 100 cells of the controls. The ratios for the width were obtained in a similar manner.

TABLE 10.—*Cell Measurements*[1]

A	Mean length, μ		Ratio $\frac{S}{C}$	Mean width, μ		Ratio $\frac{S}{C}$
	Stimulated	Control	%	Stimulated	Control	%
2352	5.512	5.88	93.7	1.107	1.092	101.4
2483	5.272	5.392	97.7	1.196	1.20	99.7
2652	4.99	5.11	97.6	1.168	1.096	106.6
2967	5.24	5.745	91.2	1.128	1.067	105.7
			95.0			103.3

[1] The mean is of 100 cells in each case from representative cultures.

A study of the tables shows that the cells in the stimulated cultures are shorter and wider than those in the control cultures. This is to be expected as the rate of the multiplication of the stimulated cultures is so much higher than that of the control cultures that the stimulated cells probably do not have time to attain the length found under normal conditions before they divide to form new cells.

Plates 3 and 4 show photomicrographs of cells from stimulated cultures and their corresponding controls made 2 years after inoculation and irradiation. The cells of the stimulated cultures are darker than those of the controls due to the greener appearance of the chlorophyll. They are also shorter and wider than the cells of the controls. More dead discolored cells are present in the control cultures than in the stimulated cultures.

CONCLUSIONS

A stimulative action causing increased cell multiplication of the green alga *Stichococcus bacillaris* Naeg. has been found to result

from sublethal exposures of the short wave lengths of the ultraviolet 2352, 2483, 2652, and 2967 A. The optimum stimulation point occurs at approximately two-thirds of the lethal exposure. The stimulative action is not transitory but has persisted in the cultures over a period of 2 years. At the end of 2 years' time, the cells in the stimulated cultures are in better condition than those in the controls. The algal cells from the stimulated cultures are slightly shorter and wider than those in the controls.

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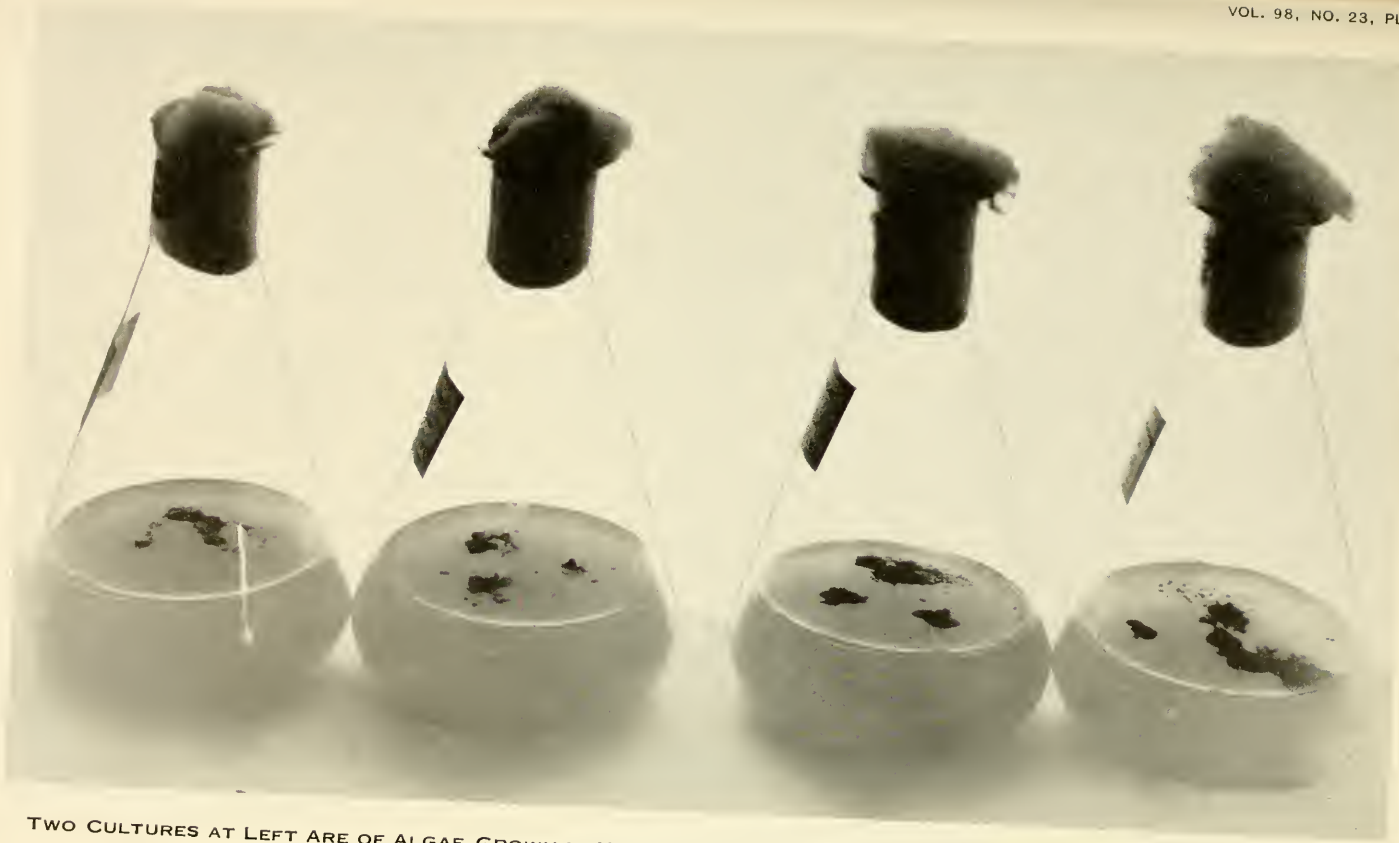
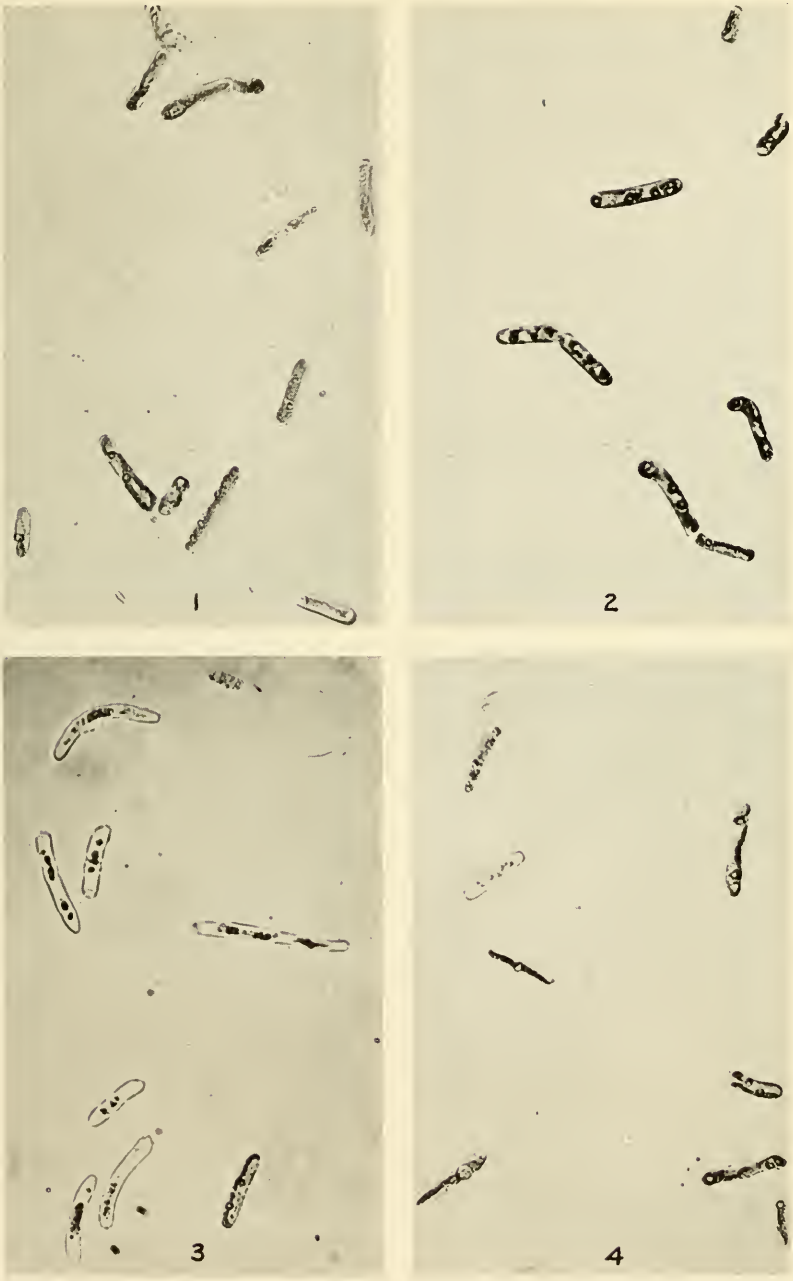


TWO CULTURES AT LEFT ARE OF ALGAE GROWN IN NATURAL CONDITIONS OF DAY AND NIGHT; TWO CULTURES AT RIGHT ARE OF ALGAE GROWN FOR THE SAME PERIOD OF TIME IN THE GROWTH CHAMBER UNDER SUNLIGHT LAMPS 12 HOURS A DAY

INTERIOR OF THE GROWTH CHAMBER



1, Algae in Control Culture; 2, Algae Irradiated 12 Minutes with 2352 A.; 3, Algae in Control Culture; 4, Algae Irradiated 100 Seconds with 2967 A. All Four Photomicrographs Were Taken 2 Years After Irradiation. x 250