SMITHSONIAN MISCELLANEOUS COLLECTIONS
VOLUME 94, NUMBER 17
(End of Volume)

GROWTH OF A GREEN ALGA IN ISOLATED WAVE-LENGTH REGIONS

(With One Plate)

BY
FLORENCE E. MEIER
Division of Radiation and Organisms, Smithsonian Institution

(Publication 3377)

CITY OF WASHINGTON
PUBLISHED BY THE SMITHSONIAN INSTITUTION
FEBRUARY 21, 1936

The Lord Baltimore Press
BALTIMORE, MD., U. S. A.

GROWTH OF A GREEN ALGA IN ISOLATED WAVE-LENGTH REGIONS

By FLORENCE E. MEIER
Division of Radiation and Organisms, Smithsonian Institution

(With One Plate)

INTRODUCTION

The importance of experimentation with isolated narrow ranges of light for the determination of the effectiveness of specific wave lengths on cell multiplication and chlorophyll formation in green algae was manifested in a previous paper by Meier (1934 b). In the research reported in that paper, 11 short wave length cut-off filters were used to transmit light of progressively shorter and shorter wave lengths from one transmitting only deep red, 6000 A, to one at the other extreme, 3700 A, where most of the visible region is included. Chlorophyll was formed in the unicellular green alga, *Stichococcus bacillaris* Naegeli, under all the filters but in best condition where the wave lengths of the blue-violet region were included. A multiplication of algal cells ranging from twofold to fourfold was obtained in the cultures. It was evident that some ranges of wave lengths seemed to inhibit cell multiplication and chlorophyll formation and that others seemed to favor them. Considering the complexity of the bands of radiation from the short wave length cut-off filters, it was clear that the effectiveness of restricted wave-length regions could be determined conclusively by experimentation in the isolated narrow ranges of light such as can be provided by Christiansen filters.

I am very grateful to Dr. C. G. Abbot, Secretary of the Smithsonian Institution, for his encouragement and helpful criticism during the accomplishment of this research. The Christiansen light filters were made by Dr. E. D. McAlister, of the Division of Radiation and Organisms, as described in his paper, "The Christiansen Light Filter: Its Advantages and Limitations" (1935). W. H. Hoover, of the Division of Radiation and Organisms, made the nephelometer measurements, as described by Meier (1934 b). Both Dr. McAlister and Mr. Hoover made the intensity measurements, and with the combined helpfulness and watchfulness of Dr. E. S. Johnston, assistant director of the Division of Radiation and Organ-

isms, L. B. Clark, and L. A. Fillmen, the successful experiments herein described were conducted under controlled conditions of temperature, humidity, and illumination.

LITERATURE

As discussed by Meier (1934 b) in more detail, some of the earlier investigators including Wiesner (1874), Artari (1899), Teodoresco (1899, 1929), Nadson (1910), and Arthur (1930), who experimented with plants growing under filters of glass and of chemical solutions, as the case may be, without especial attention to the effect of isolated wave lengths, found that their plants displayed more normal development under blue light than in the other colored lights. Teodoresco (1899, 1934) found that green light caused the poorest chlorophyll development in plants.

In his research with *Scenedesmus acutus,* growing under Senebier jars of chemical solutions, Grintzesco (1902) found that the development of the colonies was more active in the blue-violet than in the red-yellow light.

Shirley (1929) grew higher plants in a series of houses covered with glass filters transmitting definite spectral regions. He found the entire visible and ultraviolet solar spectrum to be more efficient for the growth of the plants than any small portion of it used. The blue region was more efficient than the red region.

A. Brooker Klugh (1925), using Wratten light filters whose transmission value he had very nearly equalized, found that *Volvox aureus* and *Closterium acerosum* reproduced most in red light, less in blue light, and not at all in green light.

Funke (1931) grew varieties of *Sempervivum, Ajuga,* and *Glechoma* under glass filters which let through different colored lights equalling in intensity about 25 percent of the energy of diffuse daylight. He found that the development in blue was similar to that in full daylight, red had the same influence as darkness, green produced the same phenomenon as red, or reduced development to a minimum; while in gray (subdued white light) the results were midway between those of red and blue.

Hutchinson and Ashton (1929) irradiated live specimens of *Paramoecium caudatum* in isolated wave lengths of a Hilger monochromatic illuminator operated at a low intensity of illumination. After 24 hours' exposure to the red-yellow (6152-5769 A), blue (4359-4348 A), or near ultraviolet (3663-3132 A), stimulation of growth was observed in *Paramoecia.* Retardation of growth and even death

were observed in the specimens exposed for 24 hours to the green (4968-4916 A), violet (4078-3821 A), or ultraviolet (3028-2054 A).

Johnston (1932) discusses at length the effect of infrared on the growth of plants as observed in his own experiments in addition to the conclusions formulated by other investigators. In general, it is agreed that if not actually destructive, the infrared region of the spectrum is of little or no benefit to chlorophyll formation. Burns (1933) also found infrared radiation detrimental to photosynthesis.

There exists considerable difference of opinion regarding the effectiveness of the various wave lengths. Warburg and Negelein (1923) have found the maximum chlorophyll assimilation of *Chlorella vulgaris* to be in the red (6100-6900 A), with a minimum in the blue (4360 A). Gabrielson (1935) also found assimilation in *Sinapsis alba* to be greatest in red-orange light and least in the blue-violet. Dangeard (1927), using Wratten filters, found that growth and multiplication of green algae, blue-green algae, and diatoms takes place only in red-orange light, the other radiations affecting the plants as if they were in complete darkness.

Numerous other scientists could be cited whose work gives rather contradictory results. The fact that careful scientific workers have produced contradictory evidence that leads to uncertainties emphasizes the need for precise quantitative work with isolated narrow wave length bands of light.

THE PLANT STUDIED

The unicellular green alga *Stichococcus bacillaris* Naegeli has an elongated cell usually varying from 2 to 2.5 μ in diameter and 4 to 8 μ in length. Multiplication takes place by transverse division of the protoplast which partially fills the cell and the formation of cross walls.

Cultures of this alga remain green in the dark for 2 months on Detmer $\frac{1}{3}$ agar plus 2 percent dextrose, as reported by Meier (1934 a). The best growing conditions for this alga in an artificial environment were found by Meier (1934 b) to be in Detmer $\frac{1}{3}$ solution in intermittent light when the cultures were kept agitated to favor more equal distribution of the cells, multiplication, and a more uniform lighting condition. It was also found that rubber stoppers serve as well as cotton plugs in 300 cc flasks containing 100 cc of inoculated solution for an experimental period of a month. Multiplication of this alga is proportional to the intensity of illumination, ranging from 3.76 to 34.1 microwatts/mm^2. A higher intensity such as 102.0 microwatts/mm^2 checks the growth.

APPARATUS AND TECHNIQUE

The same metal table described by Meier (1934 b) was remodeled and utilized for this experiment. (See pl. 1.) This metal table is constructed with four glass-bottomed water baths, each holding two 300 cc Erlenmeyer flasks. The four water baths are connected to a centrally located thermostated mixing chamber which kept the temperature at 19° C. In order to insure uniform dispersion of the algal cells, a common driving mechanism continually agitates the Erlenmeyer flasks. One of the cultures in each bath is illuminated from below by monochromatic light from a light filter. Mazda projection lamps served as the source of illumination. The other culture in each bath was contained in an Erlenmeyer flask which had been

TABLE 1.—*Percentage Decrease in Intensity During Each Experimental Period of 2 Weeks*

Filter	Experiments		
	2	3	4
Blue (4000-5200 A)	33.5	26.5	
Green (5000-5600 A)	50.0	27.0	
Yellow (5500-6200 A)	40.0	22.5	21.0
Red (6000-7500 A)	28.0	17.5	20.0
Infrared (8500-12000 A)			9.5 10.0

NOTE.—Intensity at beginning of each experiment was 19.5 to 20 microwatts/mm^2 for each wave-length region.

painted black to prevent the entrance of any light, thus providing a check on the culture conditions in each bath. Each of the successful experiments described here was run for a period of 2 weeks (April 9-23, May 7-21, and June 6-20, 1935), during which the temperature, humidity, and light quality were maintained constant. The cultures were given 12 hours of illumination daily, from 9: 30 a. m. to 9: 30 p. m. The cells in a drop of .01 cc volume obtained with the aid of a specially calibrated pipette and a microscope slide marked in 2-mm squares were counted microscopically for each culture at the beginning and at the end of the experiment. Nephelometric measurements of the increase of liquid turbidity as an indirect check upon the observed multiplication of cells in each culture were also made as described by Meier (1934 b). The light intensity was 19.5 to 20 microwatts/mm^2 for each wave-length region at the beginning of each experiment. The intensity dropped during each experiment as recorded in table 1.

The Christiansen filters used were constructed as described by McAlister (1935) and are shown in plate 1 on the low wooden tables by the lamps. The temperatures of the Christiansen filters were recorded twice daily to insure control of the 3 monochromatic beams utilized which were: red, yellow, and green. A Corning heat-resisting red glass filter, "205 percent", was combined with one of the Christiansen filters to give the desired region in the red. Because of the difficulty in obtaining a light source of sufficiently high intensity that would work practically with the blue Christiansen filter, it was

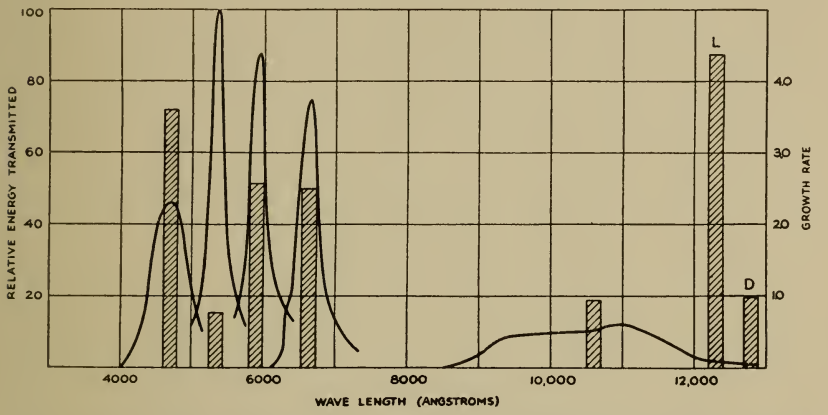

FIG. 1.—A diagram of the growth rate or cell multiplication (columns) superimposed on a diagram showing the relative energy transmitted by each filter (curves). The columns marked L and D indicate the results of daylight and darkness respectively. The abscissae indicate wave lengths. On the right is a scale of ordinates for the cross-hatched columns representing cell multiplication. On the left is a scale of ordinates representing intensities of the radiation groups. The areas under the curves representing radiation are equal, indicating the equality of radiation stimuli for visible and infrared rays. For full daylight, however, no measurement of the intensity of the radiation stimulus is available, hence the cell multiplication in daylight is not strictly comparable to the other results.

found necessary to use a saturated solution of copper sulfate as a filter. For the infrared cultures, two duplicate Corning heat-transmitting glass filters no. 254 were used. The wave-length regions for the filters are listed in the tables and shown graphically in figure 1. The daylight cultures were grown under natural conditions of day and night in a north window of a room in the Smithsonian flag tower. The dark cultures were grown in a sealed drawer of the same room.

Numerous experiments were essayed for over a year before the three successful ones reported were completed. In experiments 2 and 3, the red, yellow, green, and blue colors, as described above, were tested. The green and blue results checked in both experiments, but

as there still seemed to be some doubt as to the results of the red and yellow filters, a third experiment was run repeating the red and yellow, and with the duplicate infrared filters in place of the green and blue ones.

RESULTS

CELL MULTIPLICATION

The cells in five drops of each culture were counted and the mean was determined. As determined by the microscopic count, there was an average of seven cells in a drop of the initial cultures before treatment. The ratio of the means of the final to the initial cell count of each culture after 2 weeks of exposure to the different wave-length regions is recorded in table 2.

The nephelometric measurement, or the intensity of the light transmitted by each culture, was determined before and after each experiment. The ratio of the final to the initial measurement for each wave-length region is likewise recorded in table 2.

An examination of the cell count ratios and the nephelometric measurement ratios in table 2 shows that the check culture varies less from the treated culture of each region according to the nephelometric measurements than it does according to the cell counts. The cell counts of the check cultures show that darkness had a depressing effect on the cell multiplication, whereas the nephelometric measurements of the check cultures record the turbidity of the solution. Consequently, it hardly seems that the nephelometric measurement ratios should receive as much weight as the cell count ratios. A comparison of the two types of measurement shows that the nephelometric measurement results roughly support the cell count results.

A correction to the nephelometric indications was thought to be advisable, and was made as follows. It appeared that the nephelometric values showed a small increase of turbidity for the liquids which remained in darkness. (See table 2.) As counts showed that such a change was not caused by multiplication of algae, it seemed probably to be due to another cause, not readily ascertained, but no doubt equally operative with the flasks in which the algae were treated with radiation. Hence it seemed proper to divide the nephelometric ratios found in irradiated flasks by the mean nephelometric ratios found in the dark or check flasks before considering the nephelometric evidence as to algal multiplication under radiation. (See tables 2 and 3.)

TABLE 2.—*Growth Measurements* (*final/initial*)

	Cell count ratio						Nephelometric measurement ratio					
	Experiment						Experiment					
	2		3		4		2		3		4	
Filter	Check	Treated	Check	Treated	Check	Treated	Check	Treated	Check	Treated	Check	Treated
Blue (4000-5200 A)	0.86	3.6	1.6	3.6			1.4	2.7	1.6	2.8		
Green (5000-5600 A)	0.79	0.76	1.7	0.76			1.2	1.4	1.5	1.0		
Yellow (5500-6200 A)	0.86	2.1	1.6	2.7	1.0	2.9	2.0	2.4	1.7	2.3	1.3	2.3
Red (6000-7500 A)	0.71	1.6	1.0	3.6	0.86	2.3	1.3	1.9	1.7	3.1	1.2	2.1
Infrared					1.1	1.0					1.1	1.1
(8500-12000 A)					0.85	0.86					1.1	1.2
Daylight	0.86	4.5	1.0	4.0	1.1	4.6	1.1	3.9	1.6	4.6	1.4	4.7

TABLE 3.—*Comparison of Nephelometric Measurements with Cell Counts*

Filter	Nephelometric measurements treated/check, Experiment 2	Nephelometric measurements treated/check, Experiment 3	Nephelometric measurements treated/check, Experiment 4	Growth rate according to cell count, Experiment 2	Growth rate according to cell count, Experiment 3	Growth rate according to cell count, Experiment 4	Mean, Cell Counts	Mean, Nephelometric measurements treated/check
Blue (4000-5200 A)	1.93	1.75		3.6	3.6		3.60	1.84
Green (5000-5600 A)	1.17	.67		0.76	0.76		0.76	0.92
Yellow (5500-6200 A)	1.20	1.35	1.77	2.1	2.7	2.9	2.57	1.44
Red (6000-7500 A)	1.46	1.82	1.75	1.6	3.6	2.3	2.50	1.68
Infrared			1.00			1.0		
(8500-12000 A)			1.09			0.86	0.93	1.05
Daylight	3.55	2.88	3.36	4.5	4.0	4.6	4.37	3.26

The means for the three experiments were then determined. (See table 3.) When the means of the nephelometric results are compared with the means of the ratios of the cell counts, the same general result is obtained.

In figure 1, a diagram is given of the cell multiplication for each wave-length region superimposed on a diagram showing the relative energy transmitted by each filter. The columns marked L and D indicate results of daylight and darkness respectively. The blue region produces the greatest amount of increase over the other regions, the red and the yellow show considerable increase, the green gives a decrease, and the infrared shows no change. The daylight, the combination of all the regions of the spectrum, gives the largest increase of all. Since, however, no measurement of the intensity of the radiation stimulation was possible for daylight, the daylight results cannot be considered exactly comparable to those of the filters where the radiation stimuli are known to be equal.

The nephelometric measurement, being a very indirect method of measurement of cell multiplication, since it is based on the turbidity of the solution which doubtless undergoes a change during 2 weeks, should not be regarded as anything more than a check on the results. The cell counts give the definite result of the experiment.

CELL LENGTH

The lengths of 25 cells of each culture were measured and the means were recorded in table 4. The accuracy of the width measurements was not sufficient to show a possible increase of 20 percent. Hence, assuming no increase in width or thickness, but increased

TABLE 4.—*Cell Length (mm) at Completion of Experiments*

Filter	Experiments 2	3	4	Mean	Growth rate or cell count ratio (final/initial)	Assumed total volume
Blue (4000-5200 A)	.058	.064		.061	3.60	220
Green (5000-5600 A)	.055	.049		.052	0.76	40
Yellow (5500-6200 A)	.051	.064	.048	.054	2.57	139
Red (6000-7500 A)	.048	.053	.052	.051	2.50	128
Infrared (8500-12000 A)			.050	.050	0.93	47
Darkness	.058	.056	.056	.057	0.99	56
Daylight	.062	.054	.047	.054	4.37	236

length and multiplication of numbers as observed, the volumes of the cultures after exposure were proportional to the numbers in the final column which are the products of the mean length by the growth rate according to the cell count ratio.

These figures indicate that the blue region produced almost as large a volume as did the daylight, and one that exceeds greatly that produced by the other regions. The red and yellow regions also show a large increase in volume, while the infrared and the green cultures are less in volume than those cultures exposed to darkness.

APPEARANCE OF THE CELLS

The cells that had developed in the blue, yellow, and red regions contained beautiful green plastids, even more green than those in the daylight cultures. There were green plastids in the cells of the cultures exposed to the green wave-length region, but a great deal of granular material was also present. In the infrared cultures, the plastids were green, but there were large vacuoles and much granular material present in each cell. The cells grown in the darkness contained faded yellow-green plastids that were shriveled, broken, and in general presented a disintegrated appearance.

SUMMARY

The cell multiplication and cell length of the unicellular green alga *Stichococcus bacillaris* Naegeli were determined after 2 weeks' growth in five isolated wave-length regions of artificial light, in daylight, and in darkness.

Christiansen filters were used for the green (5000-5600 A) and yellow (5500-6200 A) regions; a combined Christiansen filter and a Corning heat-resisting red glass filter, " 205 percent ", provided the red (6000-7500 A); a saturated copper sulfate solution gave the blue (4000-5200 A); and a Corning heat-transmitting glass filter no. 254, gave the infrared (8500-12000 A). The light intensity was 19.5 to 20 microwatts/mm^2 for each wave-length region at the beginning of each experiment.

A multiplication of algal cells of over fourfold was obtained in the daylight cultures by cell counts supported by nephelometric measurements; over threefold in the blue, and over twofold in the yellow and red regions. The green region proved to be destructive, as there was a decrease in the number of cells; the infrared region made little change in cell multiplication, the cultures being very similar to those

grown in darkness. The assumed total volume based on cell length and growth rate computations varied in a similar manner.

Cells with beautiful green plastids were found in the cultures grown in the blue, red, and yellow regions as well as in the daylight. The cells exposed to the green region had green plastids but contained much granular material. Those cells exposed to the infrared had large vacuoles and very granular contents but contained green plastids. Colorless cells and cells with faded yellow-green plastids and disintegrated contents were characteristic of the cultures exposed to darkness.

LITERATURE CITED

Artari, Alexander
1899. Ueber die Entwicklung grünen Algen unter Ausschluss der Kohlensäure-Assimilation. Bull. Soc. Imp. Nat. Moscou, pp. 39-47.

Arthur, John M.
1930. Light and the green plant. Sci. Monthly, vol. 31, pp. 343-346.

Arthur, John M., Guthrie, John D., and Newell, John M.
1930. Some effects of artificial climates on the growth and chemical composition of plants. Amer. Journ. Bot., vol. 17, pp. 416-482.

Burns, G. Richard
1933. Photosynthesis in various portions of the spectrum. Plant Physiol., vol. 8, no. 2, pp. 247-262.

Dangeard, P. A.
1927. Recherches sur l'assimilation chlorophyllienne et les questions qui s'y rattachent. Le Botaniste, ser. 19, 397 pp.

Funke, G. L.
1931. On the influence of light of different wave-lengths on the growth of plants. Rec. Trav. Bot. Néerl., vol. 28, pp. 431-485.

Gabrielsen, E. K.
1935. Die Kohlensäureassimilation der laubblatter in verschiedenen spektralgebieten. Planta, vol. 23, no. 3, pp. 474-478.

Grintzesco, J.
1902. Recherches expérimentales sur la morphologie et la physiologie de *Scenedesmus acutus* Meyen. Bull. L'Herb. Boiss., pp. 217-264, 406-429.

Hutchinson, A. H., and Ashton, Miriam R.
1929. The specific effects of monochromatic light on the growth of *Paramoecium*. Can. Journ. Res., vol. 1, no. 4, pp. 292-304.

Johnston, Earl S.
1932. The functions of radiation in the physiology of plants. II. Some effects of near infra-red radiation on plants. Smithsonian Misc. Coll., vol. 87, no. 14, 15 pp.

Klugii, A. Brooker
1925. The effect of light of different wave-lengths on the rate of reproduction of *Volvox aureus* and *Closterium acerosum*. New Phytol., vol. 24, no. 3, pp. 186-190.

McAlister, E. D.
1935. The Christiansen light filter: Its advantages and limitations. Smithsonian Misc. Coll., vol. 93, no. 7, 12 pp.

Meier, Florence E.
1934 a. Colonial formation of unicellar green algae under various light conditions. Smithsonian Misc. Coll., vol. 92, no. 5, 14 pp.
1934 b. Effects of intensities and wave lengths of light on unicellular green algae. Smithsonian Misc. Coll., vol. 92, no. 6, 27 pp.

Nadson, G. A.
1910. Über den Einfluss des farbigen Lichtes auf die Entwicklung des *Stichococcus bacillaris* Näg. in Reinkulturen. Bull. Jard. Imp. Bot. St.-Pétersbourg, vol. 10, pp. 137-150.

Shirley, H. L.
1929. Influence of light intensity and light quality upon the growth of plants. Contr. Boyce Thompson Inst. Plant Res., Inc., vol. 2, no. 3, pp. 159-195.
1935. Light as an ecological factor and its measurement. Bot. Rev., vol. 1, no. 9, pp. 355-381.

Teodoresco, E. C.
1899. Influence des diverses radiations lumineuses sur la forme et la structure des plantes. Ann. Sci. Nat. Sér. Bot., ser. 8, vol. 10, pp. 141-262.
1929. Observations sur la croissance des plantes aux lumieres de diverses longueurs d'onde. Ann. Sci. Nat. Sér. Bot., vol. 11, pp. 201-336.
1934. Le développement des algues et la réfrangibilité de la lumière. Rev. Gén. Bot., vol. 46, pp. 65-74, 172-192.

Warburg, Otto, and Negelein, Erwin
1923. Über den Einfluss der Wellenlänge auf den Energieumsatz bei der Kohlensäureassimilation. Zeitschr. Phys. Chem., vol. 106, pp. 191-218.

Wiesner, Julius
1874. Untersuchungen über die Beziehungen des Lichtes zum Chlorophyll. Sitz. Math. Nat. K. K. Akad. Wiss., vol. 69, Abt. 1, pp. 327-385.

APPARATUS FOR GROWING ALGAE IN ISOLATED WAVE-LENGTH REGIONS. CONDITIONS OF LIGHT INTENSITY, TEMPERATURE, AND HUMIDITY ARE CONTROLLED IN ALL FOUR BATHS