SMITHSONIAN MISCELLANEOUS COLLECTIONS
VOLUME 87, NUMBER 10

LETHAL ACTION OF ULTRA-VIOLET LIGHT ON A UNICELLULAR GREEN ALGA

(With Two Plates)

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

(Publication 3173)

CITY OF WASHINGTON
PUBLISHED BY THE SMITHSONIAN INSTITUTION
AUGUST 17, 1932

SMITHSONIAN MISCELLANEOUS COLLECTIONS
VOLUME 87, NUMBER 10

LETHAL ACTION OF ULTRA-VIOLET LIGHT ON A UNICELLULAR GREEN ALGA

(With Two Plates)

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

(Publication 3173)

CITY OF WASHINGTON
PUBLISHED BY THE SMITHSONIAN INSTITUTION
AUGUST 17, 1932

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

LETHAL ACTION OF ULTRA-VIOLET LIGHT ON A UNICELLULAR GREEN ALGA [1]

By FLORENCE E. MEIER

Division of Radiation and Organisms, Smithsonian Institution

(With Two Plates)

INTRODUCTION

The stimulative and lethal action of ultra-violet irradiation on higher and lower plants and animals has been the subject of interesting research during the past 50 years. Unfortunately, the lack of sufficient physical data makes a correlation of the various results difficult and often inconclusive.

An accurate determination of the action of ultra-violet light on plants and animals can be obtained only by the use of monochromatic light and by measuring its actual intensity at the surface of the organisms.

For the work described here a quartz spectrograph was constructed for the purpose of exposing algae under sterile conditions to monochromatic light and thereby observing the effectiveness of a wide range of wave lengths in a definite time. This spectrograph was designed by Dr. F. S. Brackett and was constructed in the shop of the Division of Radiation and Organisms of the Smithsonian Institution. A delicate thermocouple [2] made possible the unselective determination of the relative energy of the different wave lengths.

I wish to express my gratitude to Dr. C. G. Abbot, Secretary of the Smithsonian Institution, and to Dr. F. S. Brackett, Chief of the Division of Radiation and Organisms of the Smithsonian Institution, for their aid and suggestions. The work was done with the cooperation of Dr. E. D. McAlister of the Division of Radiation and Organisms, who carried out the spectroscopic manipulations and physical measure-

[1] This paper reports investigations made under a grant from the National Research Council to the author as National Research Fellow in the Biological Sciences.

[2] The thermocouple of special design developed in the Division of Radiation and Organisms, similar to those described in the paper, The automatic recording of the infra-red at high resolution, by Brackett and McAlister, Rev. Sci. Instruments, vol. 1, pp. 181-193, 1930, was constructed by Dr. E. D. McAlister.

ments. In a paper in process of publication Dr. F. S. Brackett and Dr. E. D. McAlister will discuss more fully some of the physical problems that arise in connection with this work.

RESULTS OF OTHER INVESTIGATORS

The unicellular green algae, such as *Chlorella, Pleurococcus, Scenedesmus,* and *Chlamydomonas,* because of the similarity of their cells in size, shape, and contents when in pure culture, can be grown fairly homogeneously on a plate for exposure in the spectrograph. They thus form excellent material for the study of the effect of ultra-violet light.

As early as 1882 Engelmann placed green cells of *Oedogonium, Cladophora,* and other algae in the spectrum of a microspectroscope to observe the movement and accumulation of oxygen-loving bacteria in those regions most favorable to assimilation. He used a constant gas light and an incandescent electric light as sources of illumination. Because of the great light intensities he was able to use a narrow slit and so obtain a very pure spectrum. Ingenious as this method is, his values are only approximate. Pringsheim (1886) using the same general method found quite different values.

Ward (1893) exposed plates of agar uniformly covered with bacteria to the spectrum and then observed the behavior of the illuminated regions after incubation. He used the solar and " electric " spectra and found that no detrimental action was perceptible in the infra-red, red, orange, and yellow regions, but that all the bacteria were destroyed in the blue and violet regions and far into the ultra-violet.

Hertel (1905) was the first worker who made quantitative measurements of the intensities of monochromatic light used for ultra-violet radiation. His monochromator with its quartz prism and lenses was similar to those now used in ultra-violet microscopy. He determined the relative intensities of four lines of the ultra-violet part of the spectrum by means of a thermopile and he varied the intensity by regulating the amperage of the metallic arc. He found the region 2,800 A.[1] to have a very destructive action on paramoecia and bacteria.

In the past few years Cernovodeanu and Henri (1910), Browning and Russ (1917), Mashimo (1919), Bang (1905), Bie (1889, 1905), Bovie (1915), and a number of other workers have used the quartz spectrograph for the study of the bactericidal action of light. Raybaud (1909) made a spectrogram of three fungi. Hutchinson and Ashton (1929), and Weinstein (1930) have studied the effect of monochro-

[1] A. = Angstrom units. $1\mu = 1{,}000m\mu = 10{,}000$ A. There are 100,000,000 A. to the centimeter.

matic light on paramoecia. Hutchinson and Newton (1930) have contributed quantitative data on the effect of irradiation on yeast. Bucholtz (1931) found that the cells of higher plants are more resistant to the lethal action of ultra-violet light than bacteria and paramoecia. Weinstein (1930), Bucholtz (1931), and many of the other authors have made comprehensive reviews of the literature on ultra-violet irradiation.

Of the recent investigators, Gates (1929, 1930) has most clearly demonstrated the value of the use of monochromatic light of different intensities in the study of the lethal effect of 10 lines of the mercury-vapor spectrum on bacteria. By the use of a specially constructed monochromator and a thermopile he found the wave-length limits of the bactericidal action to be between 3,130 and 2,250 A., although the lower limit could not be positively ascertained.

EXPERIMENTAL PROCEDURE

Chlorella vulgaris, the alga which was used in this experiment, is a unicellular green alga, the spherical cell containing a parietal chromatophore and one easily visible pyrenoid. The diameter of the cell is usually 3-5μ, although some giant cells exceed 10μ. It multiplies by oval or elliptical spores, usually two to four in number. This alga has been maintained in pure culture in my collection for two years.

The nutritive solution in which the algae were grown is Detmer 1/3, 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 "
Distilled water	1. liter
Ferric chloride	a trace

Petri dishes 9.5 mm in diameter containing the above solution plus 2 per cent agar were sterilized in the autoclave at 15 pounds pressure for 20 minutes. When the media, which was about 4 mm thick, had solidified, a suspension of green cells of *Chlorella vulgaris* that had been growing in Detmer 1/3 solution in diffuse light was poured over the agar in the petri dish. This suspension of green cells was allowed to remain on the media for 24 hours, then the excess was poured off. The covered culture was placed under a bell jar and grown in diffuse light from a north window during the month of July. After a

month's time the surface of the agar plate was covered with a quite uniform green growth of algal cells.

The cover was removed, and the lower part of the petri dish was immediately covered with clear cellophane that had been soaked in 99 per cent alcohol. The culture was then placed in position in the spectrograph. It is necessary that no absorbing medium shall be present between the measured incident energy and the exposed algae. However, Johnson (1931) found that the percentage transmission of cellophane as compared to air is close to 100. Browning and Russ (1917) have demonstrated that no difference can be detected in the density of the growth of bacteria over the irradiated and non-irradiated portions of agar; consequently, ultra-violet irradiation has no appreciable effect upon agar.

After exposure of 21 minutes to the spectrum the cover of a sterile petri dish was placed over the cellophane-covered lower dish and the petri dish culture was returned to the bell jar in diffuse light.

No change was observed in the growth of the algae on this first plate until one week after exposure. Then white lines resulting from the complete decolorization of the chlorophyll and death of the green cells corresponded to the typical mercury lines for all wave lengths shorter than 3,000 A. just as they would be seen on a photographic positive (see pl. 1).

A slightly different technique was developed for the preparation of subsequent plates for exposure in the spectrograph. The surface of a glass plate of dimensions 8 by 10 cm was ground so as to retain the agar poured on it. The plate was placed in a large petri dish 15 cm in diameter and covered with Detmer 1/3 agar 2 per cent, sterilized, and inoculated as described above with a suspension of green cells of *Chlorella vulgaris*. After a month's time the agar plate covered with green cells was cut out of the surrounding agar in the petri dish and placed upright in a closed sterile brass container with a quartz window. A decker was arranged in front of the slit of the spectrograph to permit the exposure of different portions of the plate for different lengths of time.

The second plate was subjected to five irradiation periods of 6 and 20 minutes, 1, 3, and 18 hours. When the plate was removed from the spectrograph at the end of 22½ hours the effect of the 18-hour exposure was clearly visible. Three lethal regions of the 3-hour exposure were also visible. Plate 2, Figure 1 is a photograph made of the plate as soon as it was removed from the spectrograph. The algal plate was placed in a sterile petri dish in a bell jar in diffuse light. Within two days the results of all five exposures were evident.

RESULTS

Decolorized regions appeared where the plate was exposed to wave lengths 2,536, 2,652, 2,699, 2,753, 2,804, 2,894, 2,967 and 3,022 A. Those algae exposed to wave lengths 3,130, 3,341, 3,650 A. were unharmed and the cells were filled with green chlorophyll. Furthermore, it may be noted that wave lengths 3,130 and 3,650 A. are more intense by actual thermocouple measurements, as shown in Table 1.

McAlister, by the use of the double monochromator and extremely sensitive thermocouples, has accurately measured the energy distribution in the mercury arc in the ultra-violet region between 2,000 and 4,000 A. In Plate 1 the first algal spectrogram obtained in this experiment is superimposed on McAlister's record of the mercury-arc spectrum. The ordinates given here in centimeters of galvanometer deflection are proportional to the intensities measured with the double monochromator. For quantitative comparison these intensities have been corrected for the relatively lower transmission of the fused quartz system of the spectrograph used in this experiment.

Table 1 gives the intensities of the lines used and the computation of the relative lethal sensitivity to each line. Duplicate natural-color plates were made of the first algal spectrogram which was exposed for 21 minutes over the entire length of the slit. Black and white copies of these color plates were then made, and a densitometer record was determined on a Moll recording microphotometer. The curves of the density of the silver in the photographic emulsion correspond here to the algal density. A photometer record was also made of the composite superimposition of the two negatives of the color plates, the photograph of which is shown in Plate 2, Figure 2. The areas under the photometer curves corresponding to the intensity of the lethal effect were measured with a planimeter. In cases where the plates were obviously so thin that the densitometer record appears as a truncated pyramid, extrapolation to a normal curve has been made in order to correct as far as possible for this source of error. The probability is that the stronger lines are still undercorrected. The average of the areas of these three densitometer records gives the best available data for the first traverse of the color plate.

A second traverse, that is, a densitometer record across another region of the plate, was made in order to obtain a better representative determination and so minimize the inhomogeneity of this plate. The uniformity in the second traverse is such that equal weight has been given to it with the average area determinations of the first traverse.

Mean area (A) is the average of the areas from the first and second traverses. These areas should give a reasonably good measurement

TABLE 1

λ	2,536	2,652	2,699	2,753	2,804	2,894	2,967	3,022	3,130	3,341	3,650
Color plate:											
Intensity ergs/sec. cm²	480	2,050	530	480	1,930	970	2,610	5,700	12,600	1,450	28,000
First traverse:											
Negative 1 areas...	0.58	2.11	0.96	0.88	2.34	0.89	0.34	0.31	0.0	0.0	0.0
Negative 2 areas...	0.65	2.27	0.99	0.90	2.31	0.96	0.32	0.29	0.0	0.0	0.0
Composite areas ...	0.66	1.85	0.89	0.66	1.87	0.90	0.48	0.34	0.0	0.0	0.0
Average areas	0.63	2.08	0.95	0.81	2.17	0.92	0.38	0.31	0.0	0.0	0.0
Second traverse:											
Areas	0.31	1.65	0.86	0.60	1.52	0.44	0.44	0.15	0.0	0.0	0.0
Mean areas (A).....	0.47	1.87	0.91	0.71	1.85	0.68	0.41	0.23	0.0	0.0	0.0
Hg. rel. int. (B).....	2.0	8.5	2.2	2.0	8.0	4.0	10.8	23.6	52.4	6.0	115.7
Ratio $\frac{A}{B}$	0.24	0.22	0.41	0.35	0.23	0.17	0.04	0.01	0.0	0.0	0.0
Black plate:											
Intensity ergs/sec. cm².....	1,550	530	480	1,930	970	2,610	5,700	12,600	1,450	28,000	
Hg. rel. int. (B').....	6.4	2.2	2.0	8.0	4.0	10.8	23.6	52.4	6.0	115.7	
Exposure 1:											
Areas A'	0.20	0.11	...	0.27	...	...	...	...	...	...	
Ratio $\frac{A'}{B'}$	0.031	0.05	...	0.034	...	...	...	...	...	...	
Exposure 2:											
Areas A''	1.64	0.77	0.61	1.75	0.87	0.97	0.61	0.00	0.00	0.00	
Ratio $\frac{A''}{B'}$	0.26	0.35	0.31	0.22	0.22	0.09	0.03	0.00	0.00	0.00	

of the lethal effect, for they should be proportional to the algae killed, within the limitations due to plate thickness and other possible causes. If these figures are divided by the relative intensity of the lines, an approximate value is obtained of the relative lethal effect of a given quantity of incident energy of different wave lengths. These values are given in the ratio $\frac{A}{B}$. While these values have been given to two figures for the sake of uniformity, the significance of the quantities differs greatly for different wave lengths. Values for wave lengths

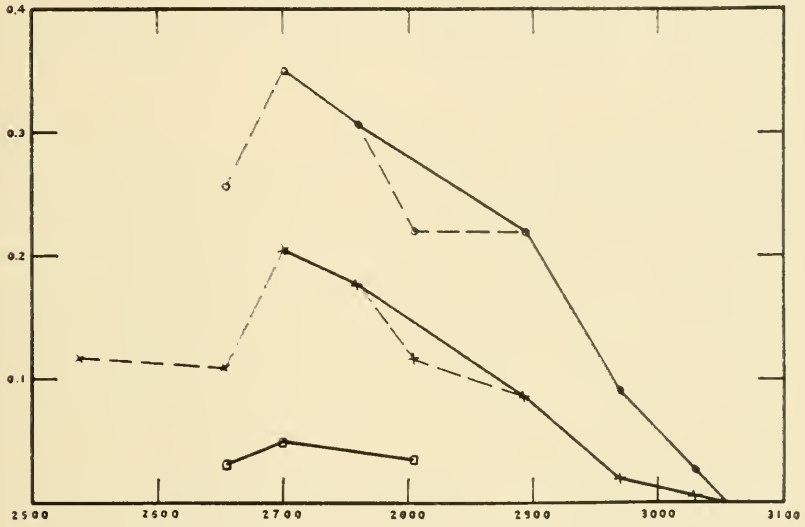


FIG. 1.—Relative lethal effect of ultra-violet light on *Chlorella vulgaris*. The ordinates are relative lethal effect in arbitrary units. The abscissae are wave-lengths in Angstrom units. (Dots occur through points of less weight.) □ Exposure 1, black plate; ○ exposure 2, black plate; × color plate.

2,536 A. should receive almost no weight because of the changing intensity due to the progressive opacity of the arc walls. Lines 2,652 A. and 2,804 A., because of the fact that they are both highly lethal and very intense, are rendered doubtful, since all the algae were killed over a wide range so that corrections for this thin plate effect are uncertain.

The black plate or second plate was taken some months later with the result that the wall of the arc had become so opaque that it was impossible to work with line 2,536 A. and the intensity of the line 2,652 A. was reduced by a quarter of its original value. Two exposures of different lengths were obtained on this plate and are shown in Plate 2, Figure 1. Exposure 1, which lasted three hours, gives an idea of the relative effects of lines 2,652 A. and 2,804 A., which are

badly overexposed when adequate exposure is made for the weaker lines. Exposure 2 lasted 18 hours and shows perceptible lethal effect even in line 3,022 A., which was scarcely noticeable in the color plate.

The values of relative lethal effect for the color plate and each of the two exposures on the black plate have been plotted and are shown in Figure 1. Those points connected by dotted lines are to be given relatively smaller weight. Of course, it should be emphasized that these measurements are only approximate in that different periods of incubation and different times of exposure may modify the relative effects of different wave lengths. As the lines differ so greatly in intensity it is hoped that further investigation can be undertaken so that the effects of intensity and time exposure may be studied.

DISCUSSION

The ultra-violet component of solar radiation at the earth's surface is from the limit of the visible spectrum, 4,000 A. to about 2,950 A. In nature, plants are exposed to invisible radiations in this region.

The amount of ultra-violet light which the plant receives varies according to the altitude, atmosphere, and season of the year. Life as it is on the earth is possible only because of the ozone formed in the upper layers of the atmosphere by the action of the short wave lengths of the ultra-violet of sunlight on oxygen. This ozone serves as a light filter and thus protects the life on the surface of the earth from the shorter destructive rays.

Throughout the ages living organisms have probably become adapted to solar radiation as it is received on the earth's surface and very possibly with the same spectrum limit due to ozone. It is, therefore, not surprising that radiation of wave lengths shorter than the solar limit produce unusual effects. While large amounts of ultra-violet of certain wave lengths are lethal, it is possible that very small amounts of the same wave lengths may be not lethal but, on the contrary, stimulating to the growth of green algae. With further experimentation we hope to obtain more definite information in regard to the possibility of this stimulative effect.

SUMMARY

It is extremely interesting to note that in the regions where the ultra-violet waves beyond 3,022 A., the approximate limit of ultra-violet irradiation in nature, were directed on the culture, the green algal cells were killed. These lethal regions appear as decolorized cells in the green algal plate at the wave lengths 3,022, 2,967, 2,894 2,804, 2,753, 2,699, 2,652, and 2,536 A. Wave lengths longer than 3,022 A.,

that is the wave lengths 3,130, 3,341, and 3,650 A., had no appreciable lethal effect upon the algae. Yet by the thermocouple measurements a greater intensity of light was directed on the cultures at wave lengths 3,130 and 3,650 A.

LITERATURE CITED

Abbot, C. G.

1911. The sun's energy-spectrum and temperature. Astrophys. Journ., vol. 34, pp. 197-208.

Bang, Sophus.

1904. Om Fordelingen af bakteriedraebende Straaler i Kulbuelysets Spektrum. Meddelelser fra Finsens Med. Lysinstitut, vol. 9, pp. 123-135.

Bayne-Jones, S., and Van der Lingen, J. S.

1923. The bactericidal action of ultra-violet light. Johns Hopkins Hosp. Bull., vol. 34, pp. 11-15.

Bie, Valdemar.

1899. Undersøgelser om Virkningen af Spektrets forskellige Afdelinger paa Bakteriers Udvikling. Meddelelser fra Finsens Med. Lysinstitut, vol. 1, pp. 33-74.

Bovie, W. T.

1915. Action of light on protoplasm. Amer. Journ. Trop. Diseases and Prev. Med., vol. 8, pp. 506-517.

1916. The action of Schumann rays on living organisms. Bot. Gaz., vol. 61, pp. 1-29.

1923. Ultra-violet cytolysis of protoplasm. Journ. Morph., vol. 38, pp. 295-300.

Brackett, F. S. and McAlister, E. D.

1930. The automatic recording of the infra-red at high resolution. Rev. Sci. Instruments, vol. 1, pp. 181-193, 1930.

1932. A spectro-photometric development for biological and photo-chemical investigations. (To be published shortly by the Smithsonian Institution.)

Browning, C. H., and Russ, Sidney.

1917. The germicidal action of ultra-violet radiation, and its correlation with selective absorption. Proc. Roy. Soc., ser. B, vol. 90, pp. 33-38.

Bucholtz, Alex. F.

1931. The effect of monochromatic ultra-violet light of measured intensities on behaviour of plant cells. Ann. Missouri Bot. Garden, vol. 18, pp. 489-508.

Busck, Gunni.

1904. Lysbiologi.—En Fremstilling af Lysets Virkning paa de levende Organismer. Meddelelser fra Finsens Med. Lysinstitut, vol. 8, pp. 7-111.

Cernovodeanu, P., and Henri, Victor.

1910. Étude de l'action des rayons ultraviolets sur les microbes. Acad. des Sci. Comptes Rendus, vol. 150, pp. 52-54.

1910. Comparaison des actions photo-chimiques et abiotiques des rayons ultraviolets. Idem, vol. 150, pp. 549-551.

1910. Action des rayons ultraviolets sur les microorganismes et sur différentes cellules. Étude microchimique. Idem, vol. 150, pp. 729-731.

Delf, E. Marion.
1928. The influence of ultra-violet light on plants. Biol. Rev. and Biol. Proc. Cambridge Phil. Soc., vol. 3, pp. 260-269.

Delf, E. Marion, Ritson, K., and Westbrook, A.
1927-8. The effect on plants of radiations from a quartz mercury vapour lamp. British Journ. Exp. Biol., vol. 5, pp. 138-153.

Ellis, Carleton, and Wells, Alfred A.
1925. The chemical action of ultra-violet rays. New York.

Eltinge, E.
1928. The effects of ultra-violet radiation upon higher plants. Ann. Missouri Bot. Garden, vol. 15, pp. 169-240.

Engelmann, Th. W.
1882. Ueber Sauerstoffausscheidung von Pflanzenzellen im Mikrospectrum. Bot. Zeit., vol. 40, pp. 419-426.
1887. Zur Abwehr. Gegen N. Pringsheim und C. Timiriazeff. Idem, vol. 45, pp. 100-110.
1888. Die Purpurbacterien und ihre Beziehungen zum Lichte. Idem, vol. 46, pp. 677-689, 693-701, 709-720.

Gates, F. L.
1929. A study of the bactericidal action of ultra-violet light. I. The reaction to monochromatic radiation. Journ. Gen. Phys., vol. 13, pp. 231-248.
1929. A study of the bactericidal action of ultra-violet light. II. The effect of various environmental factors and conditions. Idem, vol. 13, pp. 249-260.
1930. A study of the bactericidal action of ultra-violet light. III. The absorption of ultra-violet light by bacteria. Idem, vol. 14, pp. 32-42.

Hertel, E.
1905. Ueber physiologische Wirkung von Strahlen verschiedener Wellenlänge. Zeit. f. Allgem. Phys., vol. 5, pp. 95-122.

Hutchinson, A. H., and Ashton, Miriam R.
1929. The specific effects of monochromatic light on the growth of paramoecium. Can. Journ. Res., vol. 4, pp. 293-304.

Hutchinson, A. H., and Newton, Dorothy.
1930. The specific effects of monochromatic light on the growth of yeast. Idem, vol. 2, pp. 249-263.

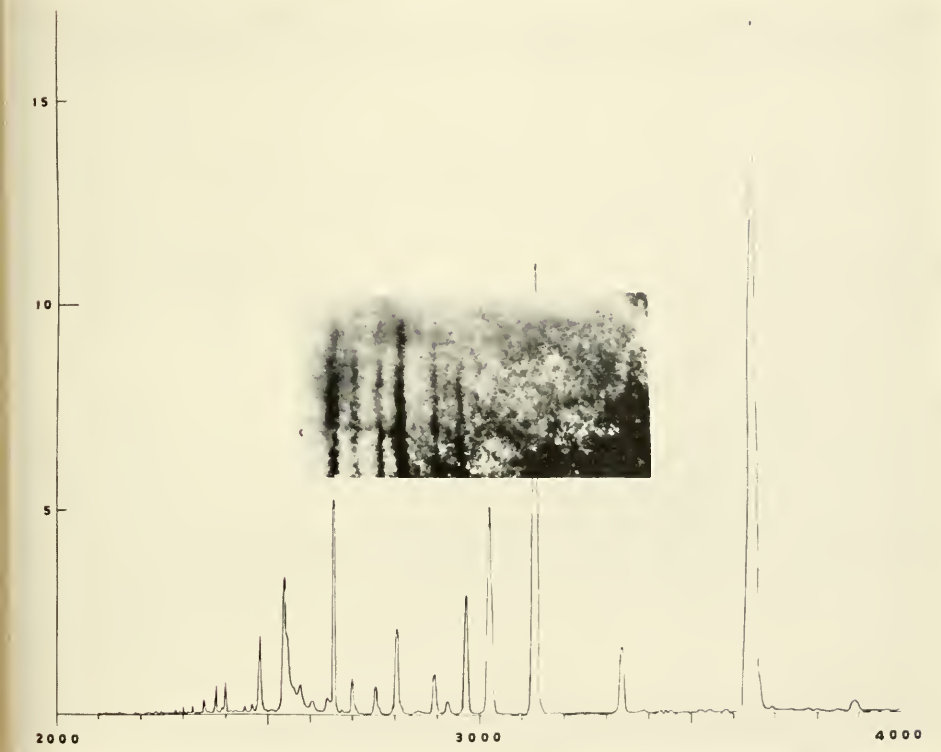
Johnson, Frank H.
1931. Cellophane covers for petri dishes for keeping out contaminations and studying the effects of ultra-violet light. Science, vol. 73, pp. 679-680.

Mashimo, Toshikazu.
1919. A method of investigating the action of ultra-violet rays on bacteria. Kyoto Imp. Univ. Col. Sci. Mem., vol. 4, pp. 1-11.

McAlister, E. D.
1929. The spectrum of the neutral mercury atom in the wave-length range from 1-2μ. Phys. Rev., vol. 34, pp. 1142-1147.

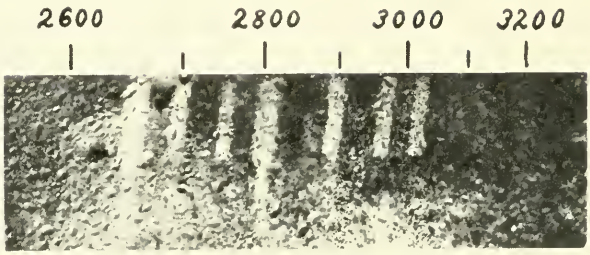
Meier, Florence E.
1929. Recherches expérimentales sur la formation de la carotine chez les Algues vertes unicellulaires et sur la production de la gelée chez un *Stichococcus* (*S. mesenteroides*). Bull. Soc. Bot. de Genève, vol. 21 (1), pp. 161-197.

NEWCOMER, H. S.
1917. The abiotic action of ultra-violet light. Journ. Exp. Med., vol. 26, pp. 841-848.
PRINGSHEIM, N.
1886. Ueber die Sauerstoffabgabe der Pflanzen im Mikrospectrum. Jahrb. f. Wissen. Bot., vol. 17, pp. 162-206.
RAYBAUD, LAURENT.
1909. De l'influence des rayons ultra-violets sur le dèveloppement des moisissures. Acad. des Sci. Comptes Rendus, vol. 149, pp. 634-636.
Report of the Secretary of the Smithsonian Institution, 1931, p. 129.
SCHULZE, J.
1909. Über die Einwirkung der Lichtstrahlen von 280 $\mu\mu$ Wellenlange auf Pflanzenzellen. Beih. Bot. Zentralbl., vol. 25, pp. 30-80.
WARD, H. MARSHALL.
1893. The action of light on bacteria. Proc. Roy. Soc. London, vol. 54, pp. 472-475.
WEINSTEIN, ISRAEL.
1930. Quantitative biological effects of monochromatic ultra-violet light. Journ. Opt. Soc. Amer., vol. 20, pp. 432-456.

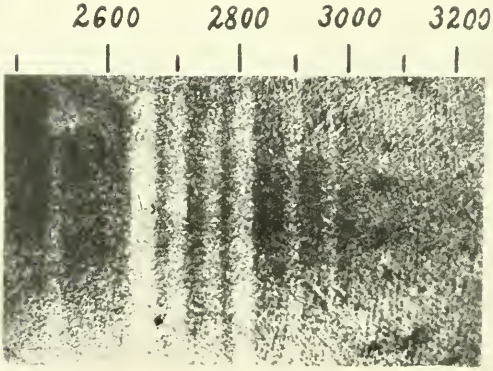


ALGAL SPECTROGRAM SUPERIMPOSED ON MERCURY ARC SPECTRUM

The ordinates are proportional to the intensity given in terms of galvanometer deflections in centimeters. The abscissae are wave-lengths in Angstrom units.



1. Black plate showing results of exposures of eighteen hours' and three hours' duration.



2. Composite of color plates showing results of exposure of twenty-one minutes' duration.

ALGAL SPECTROGRAMS