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(With Three Plates)

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

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# LETHAL RESPONSE OF THE ALGA CHLORELLA VULGARIS TO ULTRAVIOLET RAYS [1]

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## INTRODUCTION

In a previous paper Meier (1932) reported a quantitative study of the lethal effect of the wave lengths 3022, 2967, 2894, 2804, 2753, 2699, 2652, and 2536 A on the unicellular green alga *Chlorella vulgaris*. Wave lengths longer than 3022 A, which is the approximate short-wave limit of ultraviolet irradiation in nature—that is, the wave lengths 3130, 3341, and 3650 A—had no lethal effect on the green cells, although two of them, 3130 and 3650 A, were of greater intensity than the shorter lethal ones.

The present paper gives the results of further study on the lethal response of the alga *Chlorella vulgaris* to the same ultraviolet wave lengths with special reference to the radiotoxic spectral sensitivity and the radiotoxic virulence.

This 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 measurements.

I wish to express my appreciation to Dr. C. G. Abbot, Secretary of the Smithsonian Institution, for his assistance in the interpretation of the results of these experiments and for suggesting the new terms here used. I am also grateful to Dr. E. S. Johnston, Assistant Director of the Division of Radiation and Organisms, for his help in the accomplishment of this piece of research.

## RECENT INVESTIGATIONS

Striking work requiring nice technique on the lethal action of ultraviolet irradiation on certain living Protozoa has been done recently by the Cancer Research Laboratory at the University of Pennsylvania.

[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.

The micro-moving-pictures and microphotographs made by Franklin, Allen, and McDonald (1933) show how ultraviolet irradiation below 2900 A causes immediate cessation of all motion of the unicellular organisms followed by marked internal changes and in some cases a complete breakdown of the cellular structure. Swann and del Rosario (1932) noted that the death rate of the cells was not related to the intensity of the light nor to the number of the cells present, but to the length of exposure. Furthermore, cells continued to die even after the light was removed. In the work here reported it was similarly observed with algae that certain ultraviolet rays injured the cells but that death followed some time later. Swann and del Rosario found that the total number of *Euglena* cells that died subsequently as the result of irradiation was proportional to the total quantity of radiant energy in question, within the limits of intensity and concentration investigated.

The algal cells did not begin to die as soon after irradiation as did the *Euglena* cells. This may be due to a difference in the irradiation intensity. Tanner and Ryder (1923) found in their irradiation experiments that pigmented yeasts are more resistant than white yeasts and also that yeasts live a little longer than bacteria, a fact that they explain as due to the difference in size.

The work of Beauverie and Cornet (1929) on the leaf and bud of *Elodea canadensis* shows that the chloroplastids in the cell withstood continued irradiation much better than did the cytoplasm, mitochondries, and chondriocontes.

Noethling and Rochlin (1931) also irradiated *Elodea* with ultraviolet rays less than 3000 A in wave length and found a cessation of plasma streaming, also the appearance of oxalate crystals, and necrosis.

Gibbs (1926) noted that a latent period occurred before death in irradiated filaments of *Spirogyra nitida affinis*. The limits of the toxic action were the wave lengths 3126 and 2378 A. The chloroplasts were observed to clump characteristically, owing to the great difference in intensity of radiation reaching the " near " and " far " sides of the filament. The behavior of the filaments was variable. Some died while apparently perfectly normal in appearance. Coagulation of the protoplasm was noted, also a brown precipitate that exhibited Brownian movement.

Martin and Westbrook (1930) reported browning of the cells of the leaves of *Voandzeia, Pelargonium,* and other plants by ultraviolet irradiation. The browning was compared to the reddening or erythema and subsequent browning induced by ultraviolet in the human skin. Generally there is a latent period from 3 to 24 hours' duration before erythema makes itself evident. Martin and Westbrook define as the latent period the time elapsing between the irradiation and the visible culmination in browning. The term "latent period" is in a sense comparable with its application to the appearance during development of the latent image on a photographic plate.

## EXPERIMENTAL PROCEDURE

The technique, methods, and apparatus used in this work are similar to those described for the exposure of the second plate of *Chlorella vulgaris* by Meier (1932) and by Brackett and McAlister (1932).

In August 1932 three separate portions of each of 10 plates covered with Detmer $\frac{1}{3}$-agar 1.5 percent about 4 to 5 mm thick, the entire surfaces of which were uniformly green with cells of *Chlorella vulgaris,* were irradiated in the quartz spectrograph using a quartz mercury lamp as the source. Data regarding their inoculation and irradiation dates together with the exposure times are listed in table 1. In April 1933 nine additional plates (plates 14 to 17 and 19 to 23) that had been prepared in a similar fashion were also irradiated as noted in table 2. The intensity data are given in table 3.

## EFFECT OF ULTRAVIOLET RAYS ON AGAR PLATES

Two blank agar plates (plates 18 and 24) that had been made at the same time as plates 14 to 17 and 19 to 23 and left under similar conditions but not inoculated with algae were also irradiated in April 1933. Separate portions of plate 18 were irradiated for 64, 16, and 32 minutes, and separate portions of plate 24 were irradiated for 16, 4, and 8 minutes. When the plates were finally examined 2 months after inoculation, there was no evidence of any differentiated regions.

On June 24 a freshly inoculated Detmer $\frac{1}{3}$ solution of *Chlorella vulgaris* was poured over plate 18, and after a short interval the excess was removed. The plate was then placed in a north window. Within 3 weeks' time the plate was covered with a uniform green growth of the algal cells.

This experiment seems to indicate that the wave lengths which prove lethal to the green cells of the algae do not affect the culture medium which covers the glass plate in any way that will accelerate or retard the subsequent growth of the algae.

TABLE 1.—*Results of First Experiment*

| Plate | Inoculation date 1932 | Irradiation date 1932 | Irradiation time min. | sec. | Total radiotoxic regions Nov. 30, 1932 | Initial appearance of radiotoxic regions in angstroms (1932): Aug. 29 | Aug. 31 | Sept. 2 | Sept. 6 | Sept. 17 | Sept. 27 | Oct. 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | May 19 | Aug. 24 | 60 | 0 | 8 | .... .... | 2652, 2699, 2753, 2804 | 2894 .... | 2967 .... .... .... .... | 2925, 3022 | | |
| | | | 1 | 40 | 0 | | | | | | | |
| | | | 10 | 0 | 5 | .... .... | .... .... .... .... | 2652, 2804 | 2699, 2753 .... .... .... | .... .... | .... | 2894 |
| 2 | May 5 | Aug. 24 | 60 | 0 | 8 | .... .... | 2652, 2699, 2753, 2804 | 2894 .... | 2967 .... .... .... .... | 2925, 3022 | | |
| | | | 1 | 40 | 0 | | | | | | | |
| | | | 10 | 0 | 5 | .... .... | .... .... .... .... | 2652, 2804 | 2699, 2753 .... .... .... | .... .... | .... | 2894 |
| 3 | Apr. 19 | Aug. 24 | 60 | 0 | 8 | .... .... | 2652, 2699, 2753, 2804 | 2894 .... | 2967 .... .... .... .... | 2925, 3022 | | |
| | | | 1 | 40 | 0 | | | | | | | |
| | | | 10 | 0 | 5 | .... .... | .... .... .... .... | 2652, 2804 | 2699, 2753 .... .... .... | .... .... | .... | 2894 |
| 4 | June 18 | Aug. 25 | 10 | 0 | 5 | .... .... | 2652, 2699, 2753, 2804 | .... .... | .... .... .... .... .... | .... .... | .... | 2894 |
| | | | 0 | 16 | 0 | | | | | | | |
| | | | 1 | 40 | 0 | | | | | | | |
| 5 | Apr. 19 | Aug. 25 | 10 | 0 | 5 | .... .... | 2652, 2699, 2753, 2804 | .... .... | .... .... .... .... .... | .... .... | .... | 2894 |
| | | | 0 | 16 | 0 | | | | | | | |
| | | | 1 | 40 | 0 | | | | | | | |
| 6 | May 12 | Aug. 25 | 10 | 0 | Infected | | | | | | | |
| | | | 0 | 16 | | | | | | | | |
| | | | 1 | 40 | | | | | | | | |
| 7 | May 5 | Aug. 25 | 1 | 40 | 0 | | | | | | | |
| | | | 0 | 3 | 0 | | | | | | | |
| | | | 0 | 16 | 0 | | | | | | | |
| 8 | May 12 | Aug. 25 | 1 | 40 | 0 | | | | | | | |
| | | | 0 | 3 | 0 | | | | | | | |
| | | | 0 | 16 | 0 | | | | | | | |
| 9 | Apr. 19 | Aug. 25 | 1 | 40 | 0 | | | | | | | |
| | | | 0 | 3 | 0 | | | | | | | |
| | | | 0 | 16 | 0 | | | | | | | |
| 13 | Apr. 19 | Aug. 26 | 360 | 0 | 11 | 2652, 2804 | 2699, 2753 .... .... | 2894, 2967 | 2536, 2576, 2602, 2925, 3022 | | | |
| | | | 10 | 0 | 5 | .... .... | .... .... .... .... | 2652, 2804 | 2699, 2753 .... .... .... | .... .... | 2894 | |

TABLE 2.—*Irradiation Data for Second Experiment*

| Plate | Inoculation date | Irradiation date | Irradiation time, minutes |
|---|---|---|---|
| 14 | January 10, 1933 | April 17, 1933 | 8 |
| | | | 2 |
| | | | 4 |
| 15 | December 3, 1932 | April 17, 1933 | 16 |
| | | | 4 |
| | | | 8 |
| 16 | January 10, 1933 | April 18, 1933 | 32 |
| | | | 8 |
| | | | 16 |
| 17 | January 10, 1933 | April 18, 1933 | 64 |
| | | | 16 |
| | | | 32 |
| 18 | Uninoculated | April 18, 1933 | 64 |
| | | | 16 |
| | | | 32 |
| 19 | January 10, 1933 | April 18, 1933 | 8 |
| | | | 2 |
| | | | 4 |
| 20 | December 3, 1932 | April 18, 1933 | 16 |
| | | | 4 |
| | | | 8 |
| 21 | November 3, 1932 | April 19, 1933 | 64 |
| | | | 2 |
| | | | 32 |
| 22 | January 10, 1933 | April 19, 1933 | 32 |
| | | | 8 |
| | | | 16 |
| 23 | January 10, 1933 | April 19, 1933 | 72 |
| | | | 2 |
| | | | 4 |
| 24 | Uninoculated | April 19, 1933 | 16 |
| | | | 4 |
| | | | 8 |

TABLE 3.—*Intensity Data*

| A | August 23, 1932 Plates 1-13, intensity: ergs/sec. cm² | April 17, 1933 Plates 14-24, intensity: ergs/sec. cm² |
|---|---|---|
| 2536 | less than 100 | 150 |
| 2652 | 2,000 | 1,960 |
| 2699 | 680 | 640 |
| 2753 | 570 | 540 |
| 2804 | 1,930 | 1,840 |
| 2894 | 930 | 930 |
| 2925 | 450 | 440 |
| 2967 | 2,740 | 2,450 |
| 3022 | 5,700 | 5,300 |
| 3130 | 12,500 | 11,800 |
| 3340 | 1,940 | 1,720 |
| 3650 | 28,500 | 25,000 |

## RESULTS

The results of irradiating the 10 plates of the first series with ultraviolet rays are shown in table 1. The regions of decolorized cells that appeared in the green plates where the wave lengths of ultraviolet proved to be lethal or radiotoxic are tabulated with the initial dates of their appearances. The experiment was brought to an end October 30, an arbitrary date, 2 months after the irradiation date, but the plates remained in good condition until November 30 and showed no further marked differences in appearance. The total number of radiotoxic regions for each exposure was listed at this time. See plates 2 and 3.

The results of the second experiment, in which 11 plates including the uninoculated agar plates were irradiated, are given in table 4. The radiotoxicity as shown by the colorless algal regions which were present on the plates June 24, 1933, 2 months after the irradiation date, is indicated here with the exposure times. (See pl. 1.)

As indicated in tables 1 and 2, the inoculations were made from 2 to 5 months previous to the dates of irradiation. This difference in the age of the cultures had no apparent effect on the response of the algae to the ultraviolet irradiation.

### THE LETHAL RADIOTOXIC THRESHOLD

A study of tables 1 and 4 shows that the lethal radiotoxic threshold, or minimum amount of radiotoxicity required to produce lethal effect, for wave lengths 2652 and 2804 A lies between 100 and 120 seconds and probably midway between 105 and 120 seconds (the 100-second exposure being with light of greater intensity) for intensities 1,960 and 1,840 ergs/sec. $cm^2$ respectively. If it is assumed that the radiotoxic effect is proportional to the intensity and the duration of irradiation, then for 1,000 ergs/sec. $cm^2$ the exposures required for 2652 and 2804 A may be set as $1.96 \times 112 = 220$ seconds and $1.84 \times 112 = 206$ seconds respectively. For 2699 and 2753 A, 8 minutes or 480 seconds did not always suffice for killing the cells but usually did, so it is near the threshold. Then for 1,000 ergs/sec. $cm^2$, the required time would be $0.64 \times 480 = 307$ seconds and $0.54 \times 480 = 259$ seconds. Also, 2894 and 2967 A occur once or twice at 480 seconds. Hence for 1,000 ergs/sec. $cm^2$, the required time would be $0.93 \times 480 = 446$ seconds and $2.45 \times 480 = 1{,}176$ seconds. Also, injury at 2925 and 3022 A first appears at 1,920 seconds and then not always, therefore similarly we find required times of $0.44 \times 1{,}920 = 845$ seconds and

TABLE 4.—*Wave Lengths of Radiotoxic Regions Two Months after Irradiation* [a]

| Plate | Exposure time in minutes (equivalent time in seconds given in parentheses) 2 (120) | 4 (240) | 8 (480) | 16 (960) | 32 (1920) | 64 (3840) | 74 (4440) |
|---|---|---|---|---|---|---|---|
| 14 | 2652, 2804 | *2652, 2804* | 2699, 2753, | | | | |
| | .... .... | .... .... | *2652, 2804* | | | | |
| 19 | no effect | no effect | 2699, 2753, | | | | |
| | .... .... | .... .... | *2652, 2804* | | | | |
| 23 | 2652, 2804 | 2652, 2804 | .... .... | .... .... | .... .... | .... .... | 2925, 3022, |
| | .... .... | .... .... | .... .... | .... .... | .... .... | .... .... | *2652, 2699,* |
| | .... .... | .... .... | .... .... | .... .... | .... .... | .... .... | *2753, 2804,* |
| | .... .... | .... .... | .... .... | .... .... | .... .... | .... .... | *2894, 2967* |
| 21 | 2652, 2804 | .... .... | .... .... | .... .... | 2925, 3022, | 2925, 3022, | |
| | .... .... | .... .... | .... .... | .... .... | *2652, 2699,* | *2652, 2699,* | |
| | .... .... | .... .... | .... .... | .... .... | *2753, 2804,* | *2753, 2804,* | |
| | .... .... | .... .... | .... .... | .... .... | *2894, 2967,* | *2894, 2967* | |
| 15 | .... .... | 2652, 2804 | *2652, 2804* | *2652, 2699,* | | | |
| | .... .... | .... .... | .... .... | *2753, 2804,* | | | |
| | .... .... | .... .... | .... .... | *2894* | | | |
| 20 | .... .... | 2652, 2804 | 2699, 2753, | *2652, 2699,* | | | |
| | .... .... | .... .... | *2652, 2804* | *2753, 2804,* | | | |
| | .... .... | .... .... | .... .... | *2894* | | | |
| 24 [b] | .... .... | no effect | no effect | no effect | | | |
| 16 | .... .... | .... .... | 2699, 2753, | 2967, *2652,* | *2652, 2699,* | | |
| | .... .... | .... .... | 2894, *2652,* | *2699, 2753,* | *2753, 2804,* | | |
| | .... .... | .... .... | *2804* .... | *2804, 2894* | *2894, 2967* | | |
| 22 | .... .... | .... .... | 2894, 2967, | *2652, 2699,* | *2652, 2699,* | | |
| | .... .... | .... .... | *2652, 2699,* | *2753, 2804,* | *2753, 2804,* | | |
| | .... .... | .... .... | *2753, 2804* | *2894, 2967* | *2894, 2967* | | |
| 17 | .... .... | .... .... | .... .... | *2652, 2699,* | 2925, 3022, | 2925, 3022, | |
| | .... .... | .... .... | .... .... | *2753, 2804,* | *2652, 2699,* | *2652, 2699,* | |
| | .... .... | .... .... | .... .... | *2894, 2967* | *2753, 2804,* | *2753, 2804,* | |
| | .... .... | .... .... | .... .... | .... .... | *2894, 2967* | *2894, 2967* | |
| 18 [b] | .... .... | .... .... | .... .... | no effect | no effect | no effect | |

[a] The regions where the radiotoxic effect was less marked precede the more marked radiotoxic regions, which are in *italic*.
[b] Uninoculated plates.

$5.3 \times 1{,}920 = 10{,}176$ seconds. From the above rough determinations and the computation of the lethal factors for each wave length as compared with 3022 A, table 5 has been compiled and the smooth curve in figure 1 has been drawn.

TABLE 5.—*Lethal Radiotoxic Threshold and Radiotoxic Spectral Sensitivity (Based on Table 4)*

| | | Lethal radiotoxic threshold | | | |
|---|---|---|---|---|---|
| A | Intensity ergs/sec. cm² | For given intensity sec. | For 1000 ergs/sec. cm² sec. | Radiotoxic spectral sensitivity | Smooth curve |
| 2652 | 1960 | 112 | 220 | 46.3 | 45 |
| 2699 | 640 | 480 | 307 | 33.1 | 43 |
| 2753 | 540 | 480 | 259 | 39.3 | 38 |
| 2804 | 1840 | 112 | 206 | 48.9 | 34 |
| 2894 | 930 | 480 | 446 | 22.8 | 22 |
| 2925 | 440 | 1920 | 845 | 12.0 | 16 |
| 2967 | 2450 | 480 | 1176 | 8.7 | 9 |
| 3022 | 5300 | 1920 | 10176 | 1. | 1 |

## RADIOTOXIC SPECTRAL SENSITIVITY AND RADIOTOXIC VIRULENCE

The lethal response of the algae to the ultraviolet rays may be considered from two points of view, as to the radiotoxic spectral sensitivity and the radiotoxic virulence. The term "radiotoxic spectral sensitivity" relates to the certainty of the lethal action, while the term "radiotoxic virulence" may be used to describe the quickness of the attack. To make the matter clearer by analogy, let the behavior of algae with respect to three different ultraviolet rays be compared to the behavior of a human being toward three poisons, namely, radium in watch-face paint, cyanide of potassium, and rattlesnake venom.

With respect to sensitivity, each one of the three poisons is fatal if administered in a sufficient dose. Probably in order of minimum lethal dosage or sensitivity, they would rank: radium, cyanide, snake venom. But in order of toxic virulence, or the time required for lethal effect, they would rank very differently, probably: cyanide, snake venom, radium.

Applying this analogy to the selected ultraviolet rays, the determination of the radiotoxic spectral sensitivity, that is, the relative radiotoxicity of rays of different wave lengths when applied with equal intensity and duration, has been measured as described in the preceding section. The determinations for each of the eight ultraviolet rays when plotted against wave lengths gives a curve of radiotoxic spectral sensitivity. (See table 5 and fig. 1.)

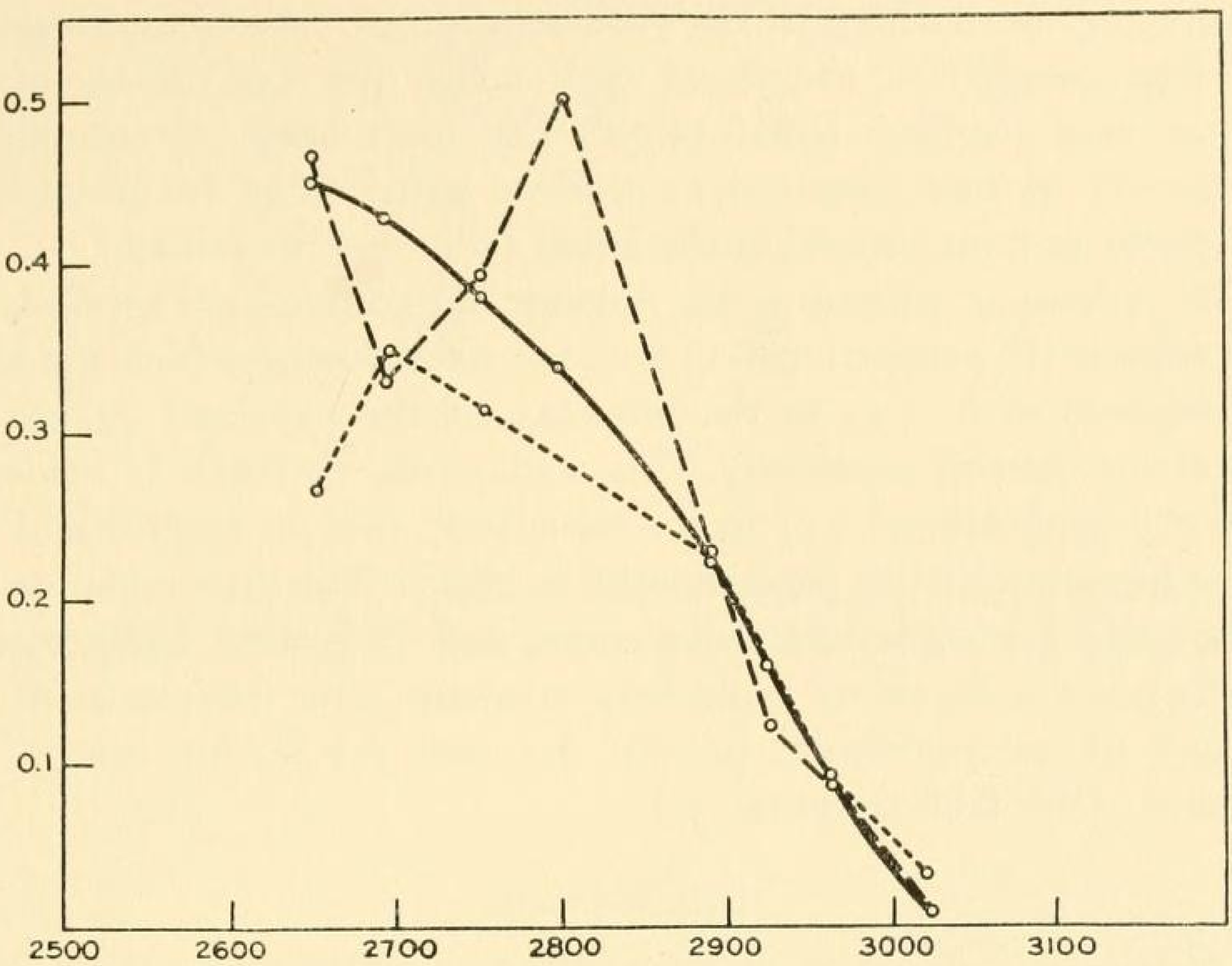


FIG. 1.—Radiotoxic spectral sensitivity of *Chlorella vulgaris* to ultraviolet rays. The abscissae are wave lengths in angstroms. The ordinates are relative lethal effectiveness in arbitrary units. Black line, smooth curve; dash line, actual values; dot line, curve obtained by Meier (1932).

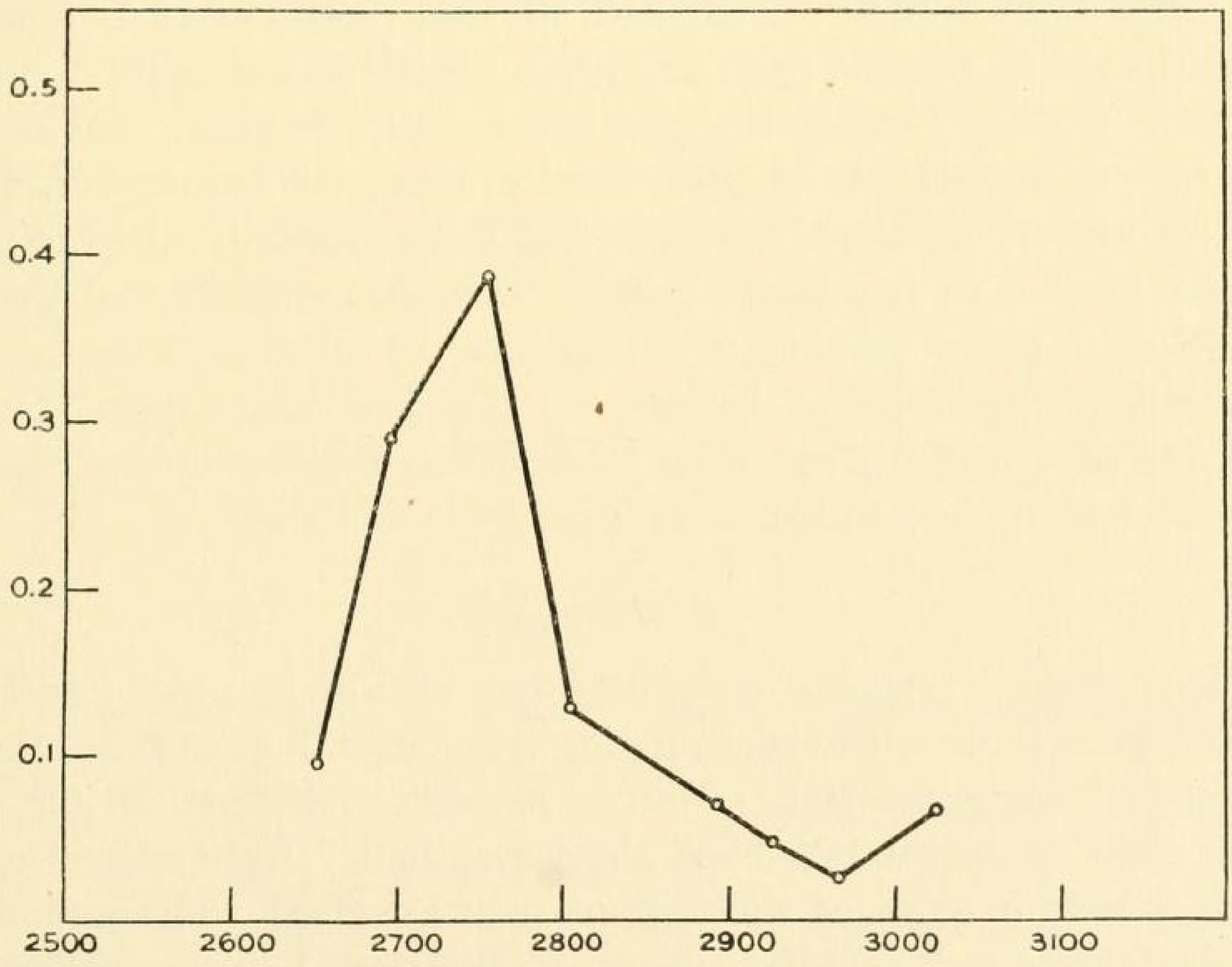


FIG. 2.—Radiotoxic virulence determined from *Chlorella vulgaris*. The abscissae are wave lengths in angstroms. The ordinates are radiotoxic virulence in arbitrary units.

Applying the analogy to the consideration of radiotoxic virulence, the determination of the speed with which the toxic doses of the several rays produce lethal effects has been made by computing the brevity of time required to produce lethal effect for a standard radiotoxic quotum exceeding the lethal radiotoxic threshold.

The radiotoxic quotum is the amount of radiotoxicity applied, and it is apparently proportional (1) to the time during which the algae are exposed to it, (2) to the intensity of the ray, and (3) to the radiotoxic spectral sensitivity. The radiotoxic virulence is evidently inversely proportional (1) to the radiotoxic quotum applied and (2) to the time required to produce a toxic effect. The determination has been made for eight ultraviolet rays, and as plotted against wave length gives a curve of radiotoxic virulence (the reciprocal of the product of the radiotoxic quotum for each ray by the time of response). (See table 6 and fig. 2.)

## DISCUSSION

The curve in figure 1 does not disagree beyond reasonable error with the one shown in figure 1 of Meier (1932), although the earlier curve was determined by a different method. It is questionable whether the wave length for the maximum radiotoxic effect has yet been determined, since effects at 2600 A and shorter wave lengths have not been sufficiently studied in these experiments. Experiments including regions 2300 to 2700 A should be made for the purpose of finding further information on this subject. Additional experiments should also be performed to check the assumption made that the radiotoxic effect is proportional to the intensity of irradiation and to the time of irradiation jointly. It is also possible that weaker irradiations would produce a stimulation of growth which is not apparent in these plates because of the luxuriant green growth covering the entire surface of each culture before irradiation. Further experiments are being planned to investigate this point.

## SUMMARY

The radiotoxic spectral sensitivity has been determined for eight wave lengths in the ultraviolet ranging from 2652 to 3022 A as applied to a unicellular green alga, *Chlorella vulgaris*. Although all the rays from 2652 to 3022 A killed the algae eventually, death ensued much more quickly in some of the regions than in others. The radiotoxic virulence or speed of effectiveness of each lethal ray in killing the algal cells for a radiotoxic quotum at eight wave lengths ranging from 2652 to 3022 A has been calculated.

TABLE 6.—*Radiotoxic Virulence (Based on Tables 1 and 5)*

| A | Exposure time min. × | Intensity × | Radiotoxic spectral sensitivity = | Radiotoxic quotum × | Days = | $\frac{\text{Product}}{100}$ | Reciprocals | Virulence[b] = $\frac{\text{Reciprocals}}{2}$ |
|---|---|---|---|---|---|---|---|---|
| 2652 | 360 | 2. | 45 | 32400. | 3 | 972. | | |
| | 60 | 2. | 45 | 5400. | 7 | 378. | | |
| | 10 | 2. | 45 | 900. | 7 | 63. | | |
| | | | | 900. | 9 | 81. | | |
| | | | | 900. | 6 | 54.[a] | 1.85 | .93 |
| 2699 | 360 | .68 | 43 | 10526.4 | 5 | 526.32 | | |
| | 60 | .68 | 43 | 1754.4 | 7 | 122.80 | | |
| | 10 | .68 | 43 | 292.4 | 11 | 32.16 | | |
| | | | | 292.4 | 13 | 38.01 | | |
| | | | | 292.4 | 6 | 17.54[a] | 5.70 | 2.85 |
| 2753 | 360 | .57 | 38 | 7797.6 | 5 | 389.88 | | |
| | 60 | .57 | 38 | 1299.6 | 7 | 90.97 | | |
| | 10 | .57 | 38 | 216.6 | 13 | 28.16 | | |
| | | | | 216.6 | 6 | 13.00[a] | 7.69 | 3.85 |
| | | | | 216.6 | 11 | 23.83 | | |
| 2804 | 360 | 1.93 | 34 | 23623.2 | 3 | 708.69 | | |
| | 60 | 1.93 | 34 | 3937.2 | 7 | 275.60 | | |
| | 10 | 1.93 | 34 | 656.2 | 9 | 59.05 | | |
| | | | | 656.2 | 6 | 39.37[a] | 2.54 | 1.27 |
| | | | | 656.2 | 7 | 45.93 | | |
| 2894 | 360 | .93 | 22 | 7365.2 | 7 | 515.59 | | |
| | 60 | .93 | 22 | 1227.6 | 9 | 110.48 | | |
| | 10 | .93 | 22 | 204.6 | 38 | 79.75 | | |
| | | | | 204.6 | 37 | 75.70 | | |
| | | | | 204.6 | 34 | 69.56[a] | 1.44 | .72 |
| 2925 | 360 | .45 | 16 | 2592. | 11 | 285.12 | | |
| | 60 | .45 | 16 | 432. | 24 | 103.68[a] | .96 | .48 |
| 2967 | 360 | 2.74 | 9 | 8877.6 | 7 | 621.43 | | |
| | 60 | 2.74 | 9 | 1479.6 | 13 | 192.34[a] | .52 | .26 |
| 3022 | 360 | 5.7 | 1 | 2052.0 | 11 | 225.72 | | |
| | 60 | 5.7 | 1 | 342.0 | 24 | 82.08[a] | 1.22 | .61 |

[a] Smallest product. [b] Halved for convenient scale.

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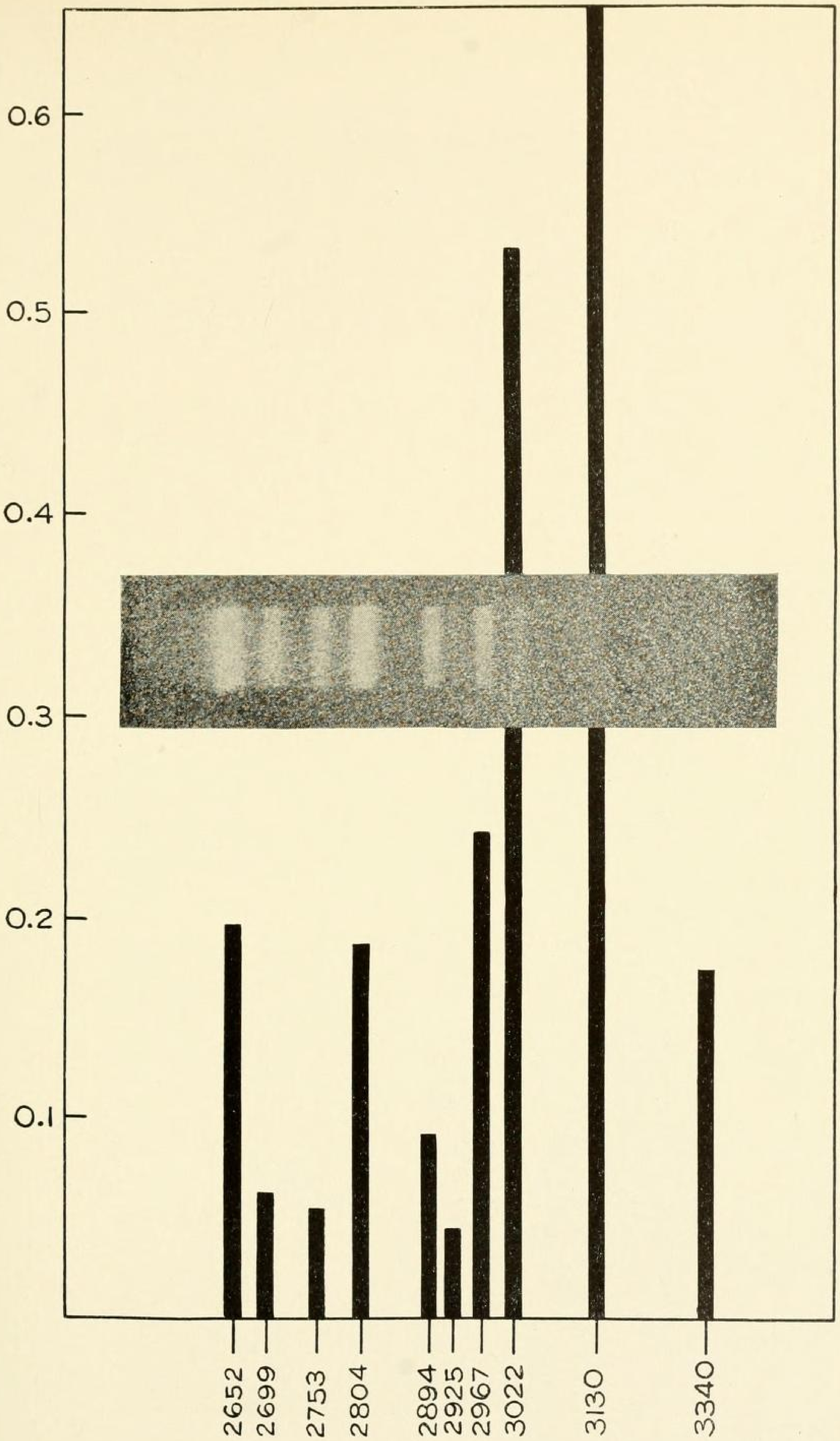
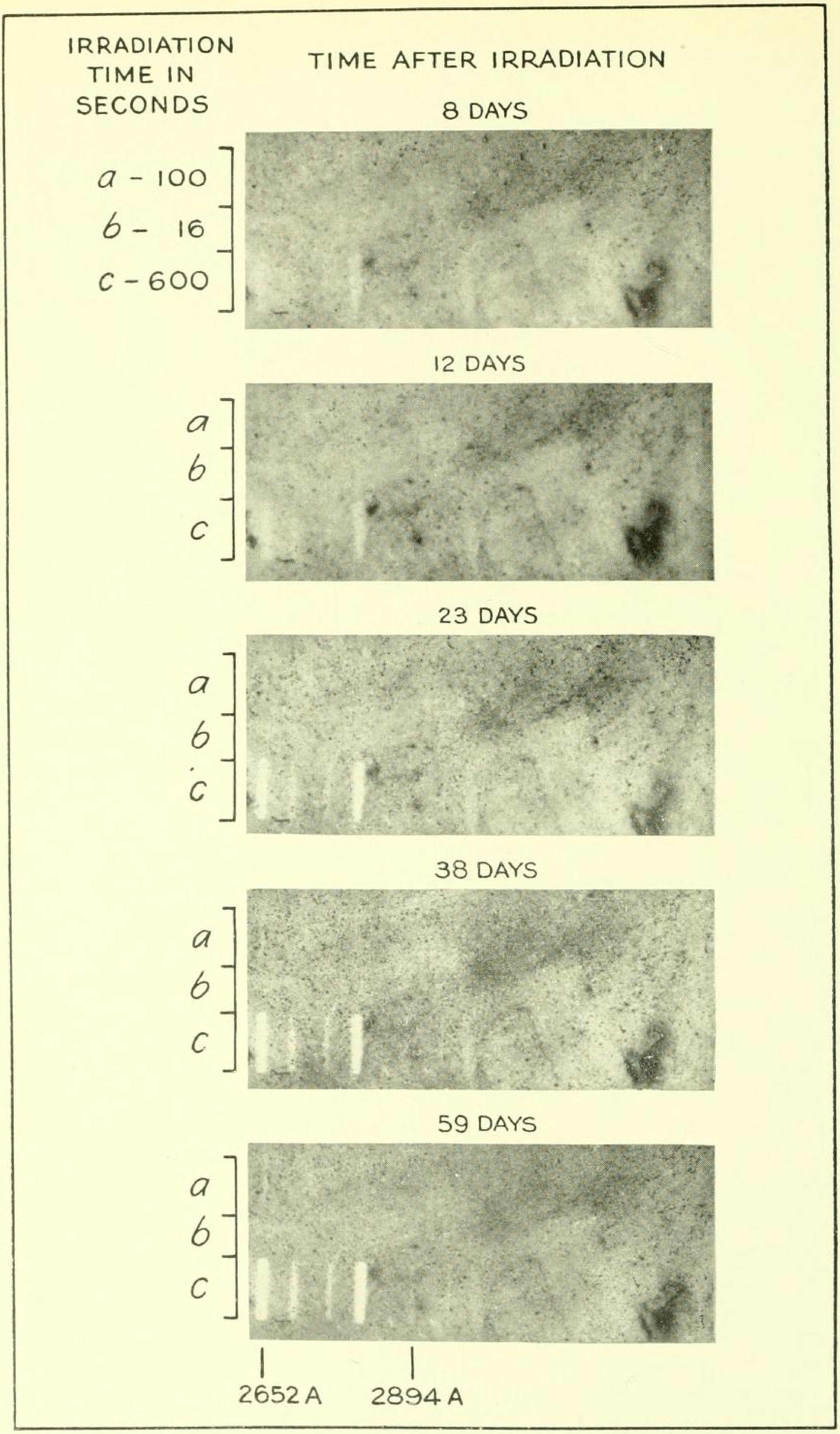


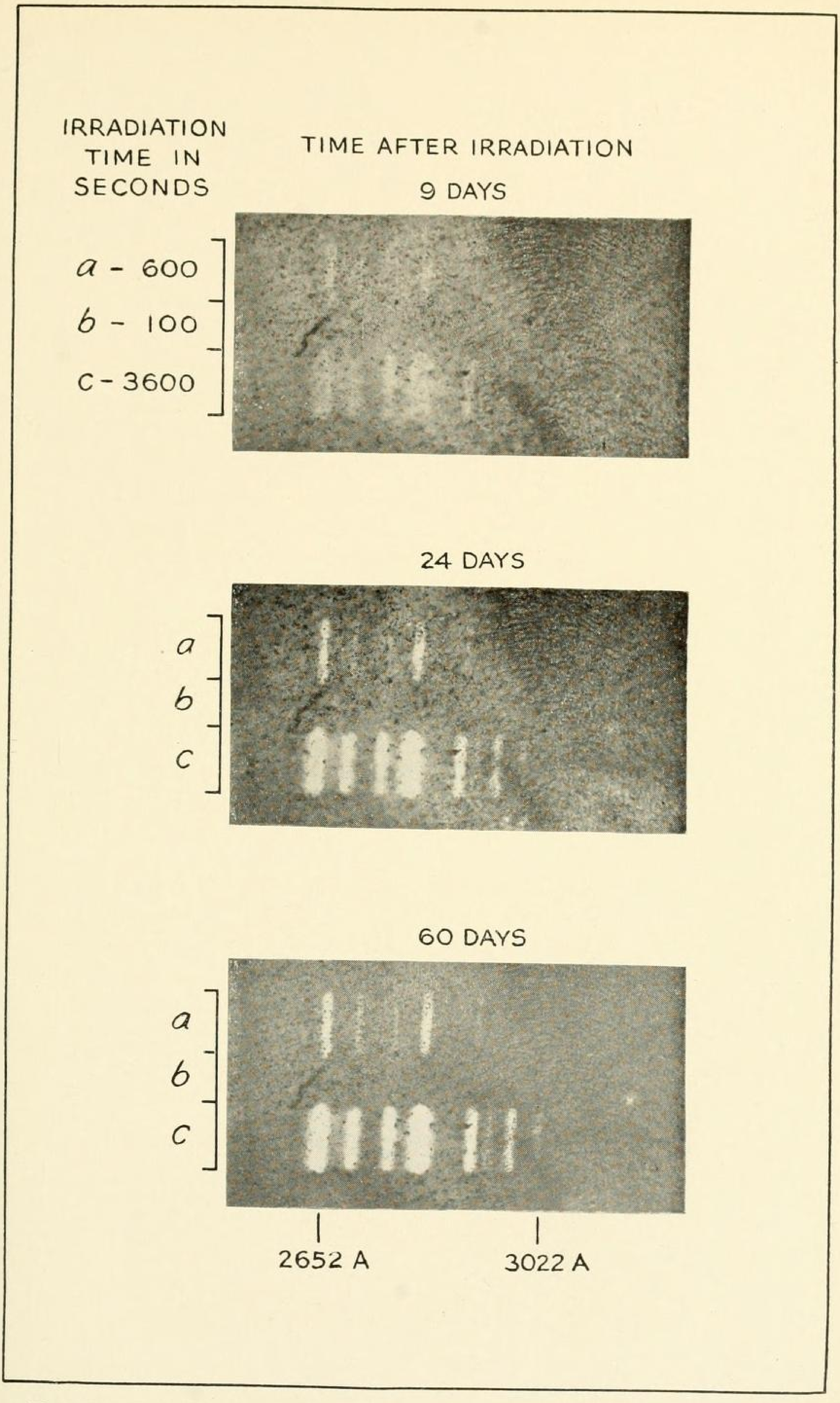

AN ALGAL SPECTROGRAM, OBTAINED BY EXPOSING PLATE 23 OF CHLORELLA VULGARIS TO ULTRAVIOLET RAYS FOR 4440 SECONDS, SUPERIMPOSED ON A DIAGRAM OF THE INTENSITIES OF THE WAVE LENGTHS.

The abscissae are wave lengths in angstroms. The ordinates are intensities in ergs/sec.cm$^2$.




GRADUAL APPEARANCE OF RADIOTOXIC REGIONS IN PLATE 5 OF CHLORELLA VULGARIS AFTER EXPOSURE OF 600 SECONDS TO ULTRAVIOLET RAYS.

No radiotoxic effect was noted for exposures of 100 seconds and 16 seconds.




RADIOTOXIC REGIONS IN PLATE 1 OF CHLORELLA VULGARIS AFTER EXPOSURES OF 600 SECONDS AND 3600 SECONDS TO ULTRAVIOLET RAYS.

No radiotoxicity was noted for the exposure of 100 seconds.

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# Viva Jesus

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Relacion historica de la creencia, usos, costumbres, y extravagancias de los Indios de esta Mision de S. Juan Capistrano, llamada la Nacion Acagchemem.

## Introduccion.

El haverme determinado á escrivir esta historia fabulosa en si, ó en lo que contiene; pero verdadera respecto de estos Indios, ha sido primeramente para poder dar en algo cumplimiento á mis obligaciones de Misionero Apostolico, teniendolo siempre presente, y la mano, como tambien dejar á mis venideros instruccion y luces para que puedan governarse sin tanto trabajo como á mi me ha costado, procurando por todos modos, empleando todos los medios posibles para adquirir el conocimiento de la creencia, usos, y costumbres, que tenian estos naturales en su gentilidad, y por la misericordia de Dios

desde 1812 con trabajo y maña en el espacio de mas de diez años, he podido averiguar

hasta 1822 con una moral certidumbre, todo quanto en el presente escrito se refiere.

Y estar persuadido de que ignorando la creencia que tienen los Indios, en sus usos, y costumbres, es muy dificil sacarlos del error en que viven, y darles á entender la verdadera Religion, y enseñarles el verdadero camino para su salvacion. Confieso que es dificil poder penetrar sus secretos, porque el significado de sus usos, y costumbres no lo saben todos, esto es solo para los Capitanes y algunos Sahapas, que hacian el oficio de Sacerdotes, y pregoneros, y quando estos lo enseñavan á sus hijos (y esto solo á los que los havian de suceder) era siempre con la advertencia que no lo manifestáran á nadie, porque si lo decian ó manifestavan tendrian muchas desgracias, y que se moririan infundiendoles mucho temor y miedo y por tanto se sabe tan poco de sus cosas, porque los pocos que lo saben y entienden lo tienen reservado para si.

REPRODUCTION OF PAGE 1 OF THE NEWLY DISCOVERED MANUSCRIPT