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THE DELAYED LIGHT AND FLUORESCENCE EMISSION SPECTRA FOR GREEN, BLUE-GREEN, RED ALGAE, AND NONPHOTOSYNTHETIC MUTANTS

J. R. Azzi

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THE DELAYED LIGHT AND FLUORESCENCE EMISSION SPECTRA FOR GREEN, BLUE-GREEN, RED ALGAE, AND NONPHOTOSYNTHETIC MUTANTS

J. R. Azzi

AUGUST 1966

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THE DELAYED LIGHT AND FLUORESCENCE EMISSION SPECTRA FOR GREEN, BLUE-GREEN, RED ALGAE, AND NONPHOTOSYNTHETIC MUTANTS

J. R. Azzi

SUMMARY

(1) The delayed light and fluorescence emission spectra of *Chlorella* have been reinvestigated, and the similarity of the two spectra has been reconfirmed; that is, the observable emissions are coming from excited chlorophyll. The peaks of the emissions are at 685 mμ.

(2) The delayed light emission spectrum of *Anacystis* is essentially the same as that of *Chlorella*, with the emission coming from excited chlorophyll, and the peak of the emission is at 675 mμ.

(3) The fluorescence emission spectrum of *Anacystis* is not the same as the delayed light spectrum, since the phycocyanin emission can be observed as well as the chlorophyll emission. The phycocyanin emission can be intensified by exciting the cells with sodium light.

(4) The delayed light emission spectrum for *Porphyridium* is essentially the same as that of *Chlorella*, and the peak of the emission is at 680 mμ.

(5) The fluorescence emission spectrum for *Porphyridium* is not the same as the delayed light spectrum, for the accessory pigments phycoerythrin and phycocyanin are clearly emitting in the shorter wavelength region.

(6) The delayed light and fluorescence spectra for *Scenedesmus* wild type are the same as those of *Chlorella*, with the peaks of the emissions at 685 mμ.

(7) The delayed light emission spectrum for mutant 8 is essentially the same as that in both the wild type and *Chlorella*, with the peak of the emission at 685 mμ.

(8) The fluorescence emission spectrum for mutant 8 shows a higher emission in the long wavelength region as compared with fluorescence spectra for the wild type and mutant 11. As stated in the text, this mutant does not contain any measurable P-700.

(9) The fluorescence emission spectrum for mutant 11 is the same as that of both the wild type and *Chlorella*, with the peak emission at 685 mμ.

(10) From other experiments done in this laboratory (unpublished), the delayed light signal at 200 cycles per second from mutant 11 is 300 times less in intensity than the delayed light signal from the wild type. This signal was too small for our detection apparatus to give a delayed light emission spectrum for mutant 11.

I. INTRODUCTION

In 1951 a communication from this laboratory[1] described a light emission by green plants which lasted for some seconds after illumination. Since that time this delayed light has been under investigation in the hope that it will lead to a better understanding of the first steps in photosynthesis.

The emission spectrum of the delayed light is basic to any understanding of the phenomenon, but the low intensity of the emission makes it difficult to obtain spectra showing much detail. However, earlier published spectra[2,3] for *Chlorella* and spinach chloroplasts showed that the delayed light and fluorescence emission spectra were very similar, and that the delayed light must come from excited chlorophyll. In 1956,[4] emission spectra were published for the delayed light from blue-green and red algae. The data indicated that the delayed light came only from chlorophyll, while the fluorescent light came from the accessory pigments, phycocyanin and phycoerythrin, as well as from chlorophyll.

At the time the above experiments were done, it was not realized that the intensity of the delayed light continued to increase as the time between illumination and measurement was decreased below $\frac{1}{2}$ sec.[5,6] The construction of a new phosphoroscope that allows measurements of delayed light at times of a few milliseconds and that will fill our *f*/4 monochromator with light makes it now possible to repeat the emission spectra for the delayed light from the green, the blue-green, and the red algae, with greatly improved precision. In addition to repeating the older experiments, we have determined the emission spectra for the wild type and the nonphotosynthetic mutants of the green alga, *Scenedesmus*, isolated by Bishop.[7,8]

Scenedesmus "O_2" mutant 11 is believed to have photoreaction II blocked and the "CO_2" mutant 8 is believed to have photoreaction I blocked along with the absence of P-700.[9,10] We felt that these mutants could possibly show important spectral differences which could further elucidate the two photoreaction hypotheses.

II. MATERIALS AND METHODS

Biological Material

The stock cultures of *Chlorella*, *Anacystis*, and *Porphyridium* were obtained from the Culture Collection of Algae, Indiana University. The *Scenedesmus* wild type and mutants 8 and 11 were obtained from Norman I. Bishop, Department of Botany, Oregon State University, Corvallis, Oregon.

Chlorella pyrenoidosa, Emerson's strain "252," was grown at 20°C in Knop's medium under neon lamps and was bubbled with a mixture of CO_2 (5%) and air (95%).

Anacystis nidulans was grown at 36°C in *Anacystis* medium C[11] under a sodium lamp (General Electric NA-9) and was bubbled with CO_2 (5%) and air (95%).

Porphyridium cruentum was grown at 20°C in Jones' artificial seawater[12] under fluorescent lamps covered with a layer of yellow cellophane and was aerated.

Scenedesmus obliquus, Gaffron's strain D_3, wild type, and x-ray mutants 8 and 11 were grown at 20°C in Bishop's organic medium[13,14] in the dark, shaking at a frequency of 50 cycles per minute.

The concentration of cells was determined by either of two methods, and the concentration units are given in the figure legends. The hematocrit method (centrifugation of cells for 5 min at 500 × *g* in a hematocrit tube, which measures mm^3 of cells per 10 cc suspension) was used for *Chlorella, Porphyridium,* and *Scenedesmus*. Because of their small size, *Anacystis* could not be accurately centrifuged and measured by the hematocrit method; therefore, a determination of percent transmission of *Anacystis* at 800 mμ on a Beckman spectrophotometer was the method used.

Preliminary experiments were done to determine an optimum concentration of cells for each alga used for the two spectra. These results were plotted as delayed light intensity at 690 mμ vs cell concentration. We chose the highest cell concentrations on the linear part of the curve, and this established an important condition for the spectral experiments; that is, to have maximal signals without distortion in the shape of the spectral curve due to self-absorption by using a too concentrated cell suspension.[2]

III. EXPERIMENTAL APPARATUS

A modified Becquerel phosphoroscope was used for the spectral experiments and is described in Fig. 1. An RCA-7102 photomultiplier was used to observe the light emission from the various algae, and it is described in Fig. 2.

The components of the apparatus are schematically represented in Fig. 3. The signal from the photomultiplier was monitored by a vibrating-reed electrometer with a 6-sec time constant used as a smoothing circuit, then on to a Brown recorder.

The delayed light emission spectra were taken when the shutters rotated at 200 cycles per second. The exciting light consisted of a 1000-w tungsten lamp, its filament focused on the cell suspension to give an image of approximately 1 in. square, and the bulb's incandescent emission was filtered through 10 cm of water.

The exciting light for the fluorescence emission spectra was either a 1000-w tungsten lamp, which was filtered through 10 cm of saturated $CuSO_4$, or a sodium Lab-Arc with filter 9782 in the exciting light beam. The intensity of the exciting light was adjusted to give a fluorescent signal a little larger than the delayed light.

IV. SPECTRAL MEASUREMENTS

Collection of the delayed light and fluorescence emission data was started after the dark-light transients were diminished. With the transients over and with the 6-sec time constant, the signal at each wavelength would reach a plateau in 30 sec. Therefore, each wavelength measurement was taken for 30 sec, and the cells were illuminated continuously so that the transients did not reoccur during the experiments.

As a starting reference, the first wavelength measurement was taken at 680 mμ, then taking the even increments of 10 mμ from the short wavelengths up to 800 mμ partially completed the spectrum. The 680 mμ was repeated as a check on reproducibility, then taking the odd increments of 10 mμ from 755 mμ down to the short wavelengths completed the spectrum with wavelengths 5 mμ apart. A final setting at 680 mμ was recorded as a check on any changes that occurred to the cell suspension in the 30 to 40 min to make the spectrum. All experiments were done at room temperature, 22 to 25°C.

V. RESULTS

The signal at each wavelength of the delayed light and fluorescence emission spectra was read directly from the Brown recorder chart. In order to have the signal represented as relative energy per unit wavelength, the data were corrected for the linear dispersion of the monochromator and for the sensitivity of the photocell (Fig. 4). The corrected spectra were normalized at the peak wavelength emission to full scale and plotted as relative energy per unit wavelength vs wavelength.

The results are graphically represented by the delayed light and fluorescence emission spectra for each alga studied, along with a brief description of each curve in the figure legends.

VI. ACKNOWLEDGMENTS

This investigation was suggested by William Arnold and J. B. Davidson, and the author is grateful to them for their criticism and guidance, and to Michael Kamrin for suggestions concerning the problem.

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Fig. 1. Schematic Diagram of a Modified Becquerel Phosphoroscope. The entrance and exit apertures for the exciting light and emitted light respectively are 1 in. long and $\frac{5}{16}$ in. wide. These apertures were chosen in order to fill the Farrand monochromator, model 300 VIS, which has an aperture of *f*/4.

The apparatus consisted of two balanced shutters mounted on a common shaft. The shutters rotated in narrow slits of the aluminum housing. The shutter diagrammed in Fig. 1 has two open segments that are exactly opposite and 70° wide. With the front shutter open, the cell suspension was illuminated, while the back shutter was closed, thus protecting the photomultiplier from the bright exciting light. When the front shutter closed, the preilluminated cells emitted delayed light through the now open back shutter which allowed the cells to be viewed by the photomultiplier for a period between each exciting flash.

On the side of the phosphoroscope perpendicular to the entrance and exit apertures and located between the two shutters was another entrance aperture with a diameter of 1 in. (Fig. 3). The cells were illuminated through this aperture with the back shutter open so that the photomultiplier could observe the fluorescence emission of the cells.

The sample holder, a cellulose nitrate tube No. 661, positioned between the two shutters contained 36 ml of cell suspension. The shutters and interior walls of the aluminum housing were painted with flat black paint (3M Co., Velvet Coating No. 9564).

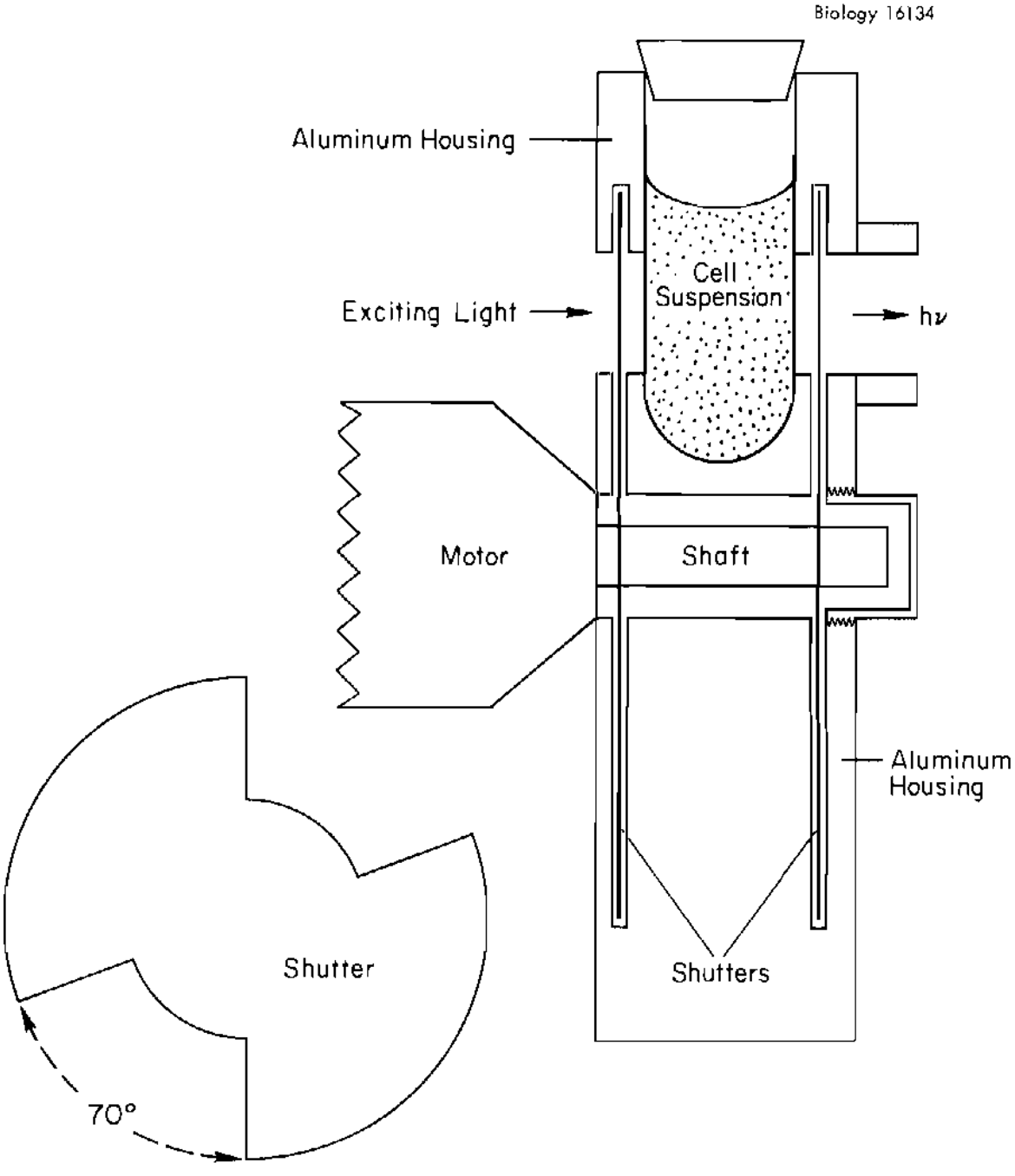

Biology 16134
Aluminum Housing
Cell Suspension
Exciting Light
hν
Motor
Shaft
Aluminum Housing
Shutter
Shutters
70°

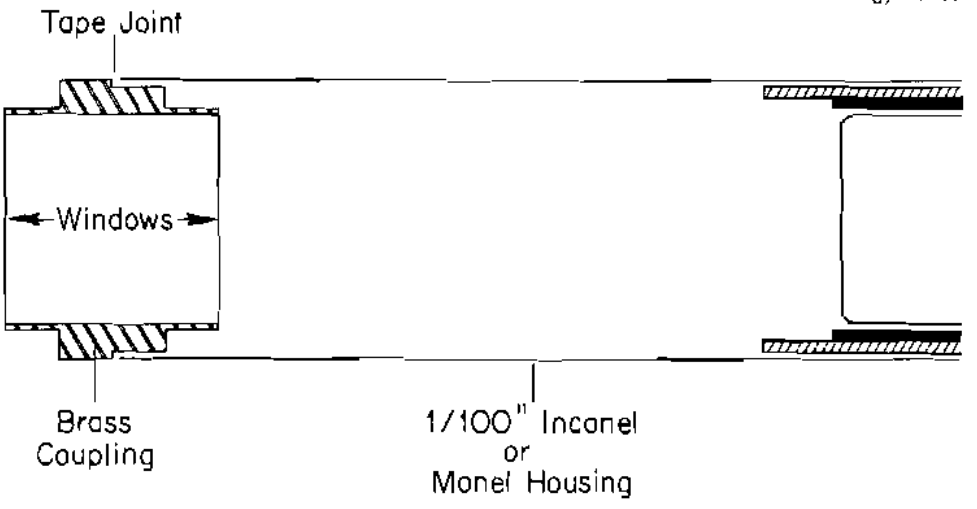
Fig. 2. Schematic Drawing of the Photomultiplier Tube RCA-7102 Enclosed in an Inconel Housing. The 7102 photocell is a 10-stage, head-on type with an S-1 response. The apparatus was inserted into a styrofoam cooler and surrounded with dry ice. This system of cooling the photocathode lowered its dark current, thus increasing its efficiency and sensitivity.

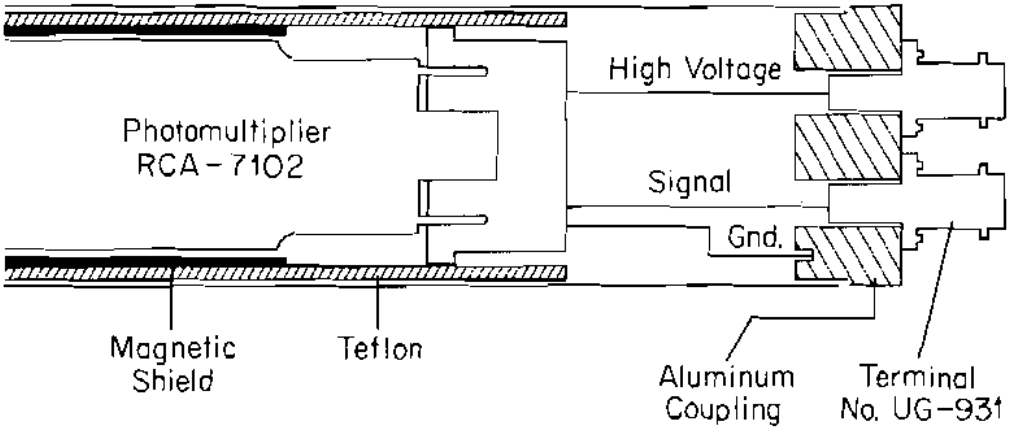
The high-voltage, signal, and ground leads were of constantan wire No. 40. By using this wire and the Inconel metal (1/100 in. thick), we could have the two ends of the photomultiplier housing at room temperature while the photocell remained cold, for there is practically no heat flow through these very thin materials.

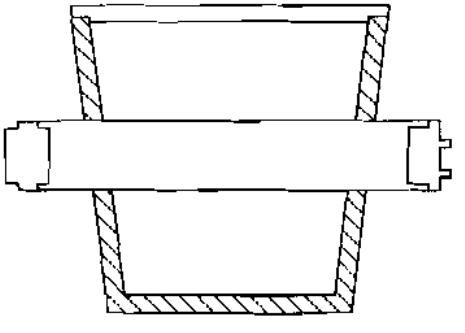
The magnetic shield is a cylinder of Mumetal No. 80802-C (James Millen Manufacturing Company, Malden, Mass.). The magnetic shield was electrically connected to the photocathode and wrapped with a layer of teflon, then inserted into the inconel housing.

The front windows were of Saran Wrap, and the dry air space between the windows prevented frost from forming on them. A jet of air was passed over the ends protruding from the cooler in order to keep frost from forming on the tape joints and terminals. In order to keep light from leaking into the photomultiplier housing, the brass and aluminum couplings were tightly fitted into the housing, and the joints were sealed with several layers of vinyl plastic electrical tape.

Biology 16135





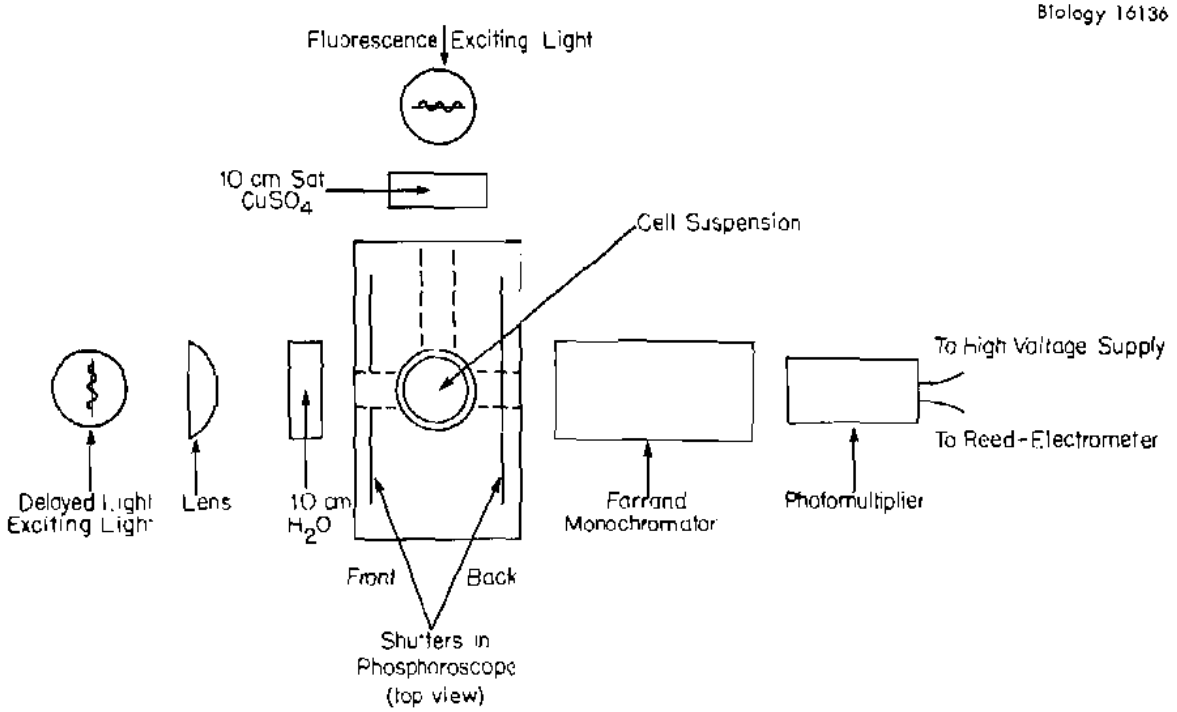


Photomultiplier Unit
Mounted in Styrofoam Cooler

Fig. 3. A Block Diagram of the Components Involved in Obtaining Delayed Light and Fluorescence Emission Spectra. The phosphoroscope, monochromator, and photomultiplier were connected to each other with light-tight seals of vinyl electrical tape.

The delayed light spectra were taken when the phosphoroscope shutters rotated at 200 cycles per second. For the fluorescence emission, the cells were illuminated from the side with the back shutter open (front shutter would be closed).

Biology 16136



Schematic Diagram of Apparatus

Fig. 4. The Photocell Sensitivity Curve. From RCA-7102 specifications, obtained from Electron Tube Division, RCA, Harrison, New Jersey. The linear dispersion curve is from a Forrand manual, obtained from Forrand Optical Company, New York, N.Y.

The signal read from the Brown recorder chart was corrected by the following formula: $J \approx S/D_\lambda \cdot P_\lambda$, where J is the corrected emission in relative energy per unit wavelength, S is the experimental data (signal), D_λ is the linear dispersion of the monochromator in millimicrons per millimeter, and P_λ is the photocell sensitivity in arbitrary units.

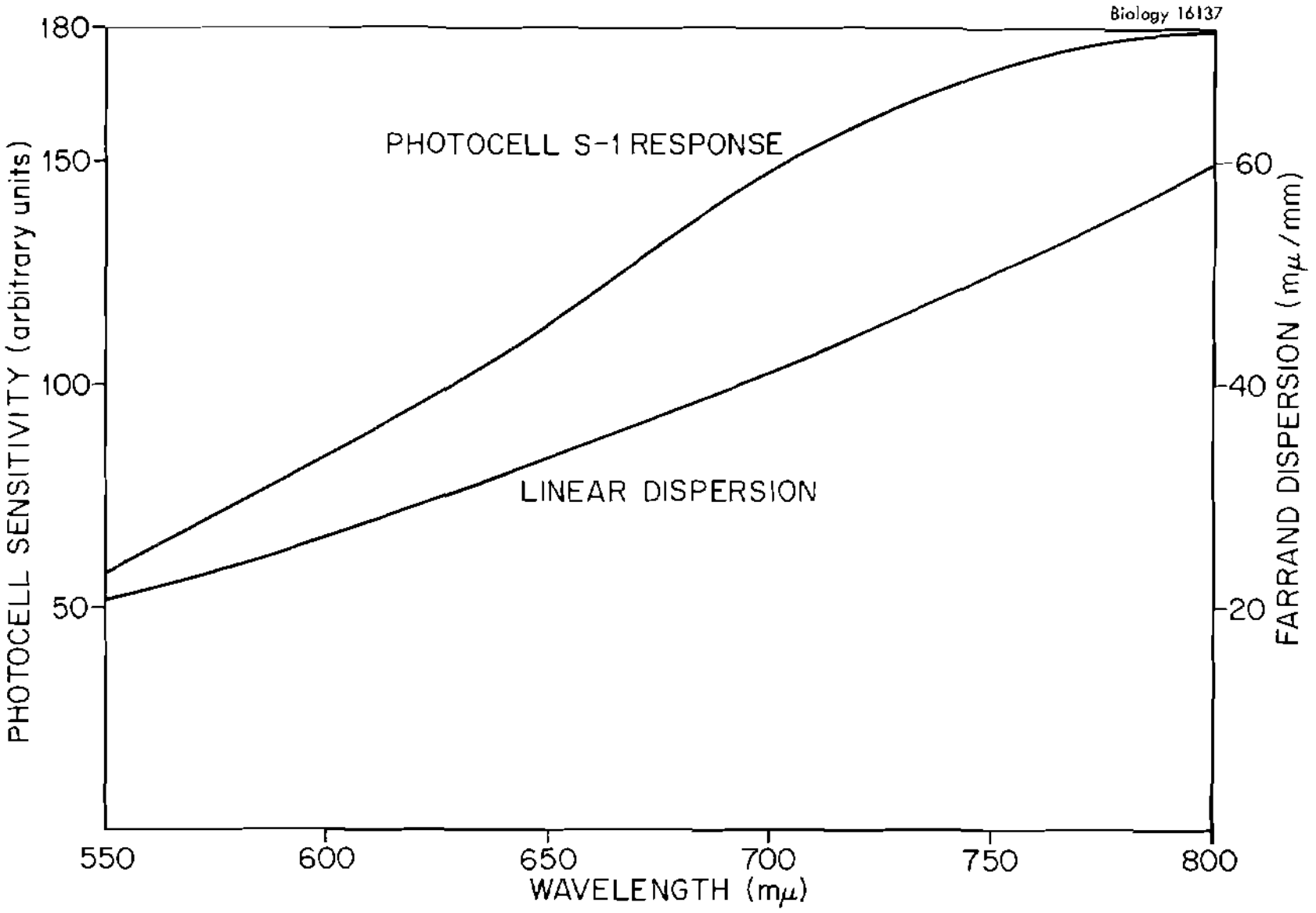

Biology 16137
PHOTOCELL S-1 RESPONSE
LINEAR DISPERSION
PHOTOCELL SENSITIVITY (arbitrary units)
FARRAND DISPERSION (mμ/mm)
WAVELENGTH (mμ)
180
150
100
50
60
40
20
550
600
650
700
750
800

Fig. 5. *Chlorella* Fluorescence Emission Spectrum (●—●). Cells 3 days old set at a concentration of 5 $mm^3/10$ cc; blue light excitation; slit widths in millimeters (0.1 entrance, 0.17 exit). *Chlorella* delayed light emission spectrum (o--o). Cells 4 days old set at a concentration of 4 $mm^3/10$ cc; white light excitation; slit widths same as above.

As previously reported by Arnold and Davidson in 1954, the present experiments for *Chlorella* also show that the delayed light and fluorescence emissions are coming from excited chlorophyll with the wavelength peak of 685 mμ.

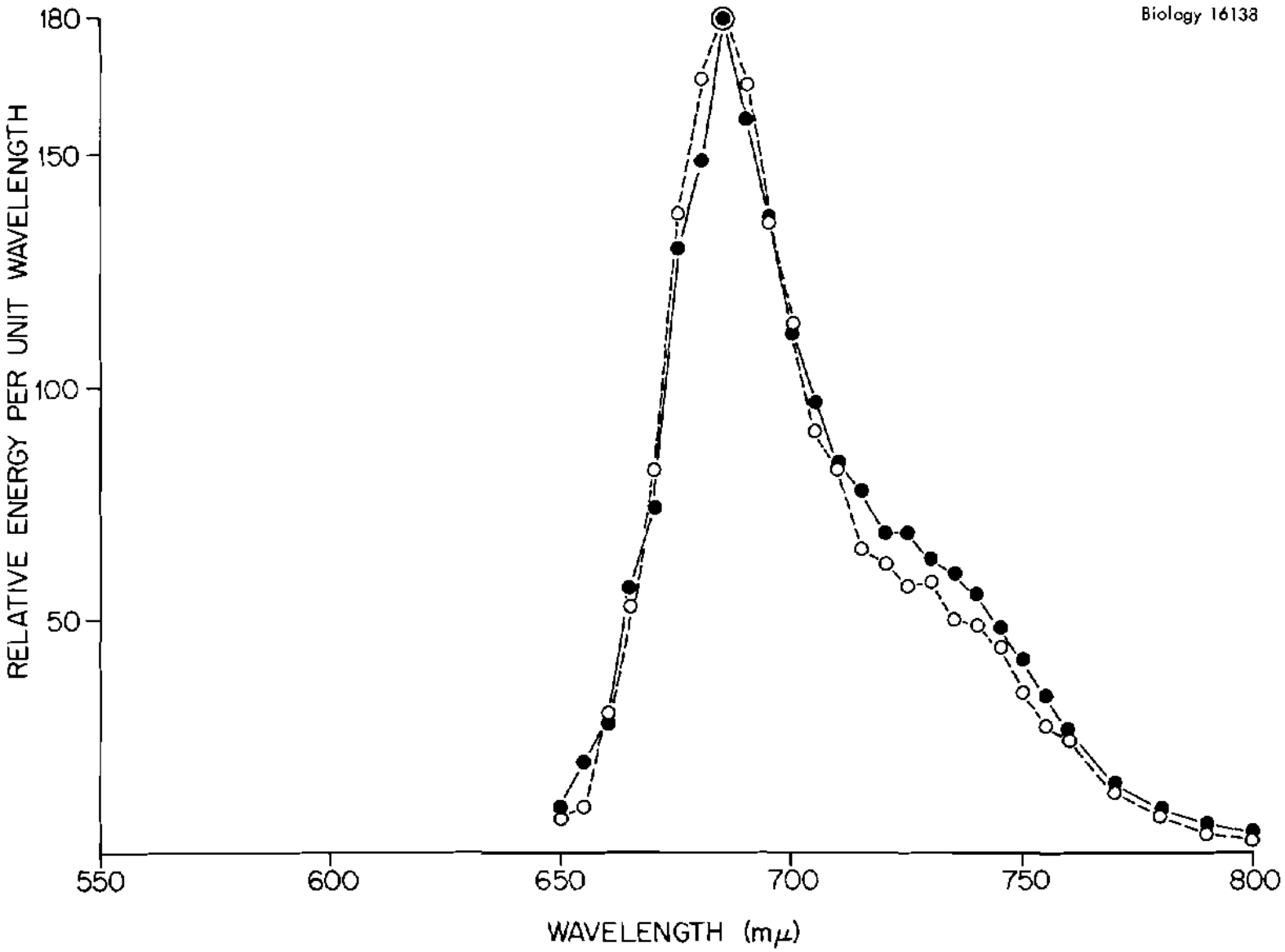

Biology 16138
RELATIVE ENERGY PER UNIT WAVELENGTH
180
150
100
50
550
600
650
700
750
800
WAVELENGTH (mμ)

Fig. 6. *Anacystis* Fluorescence Emission Spectrum (●—●). Cells 2 days old set at 80% transmission at 800 mμ; blue light excitation; slit widths in millimeters (0.24 entrance, 0.31 exit). *Anacystis* delayed light emission spectrum (o--o). Cells 2 days old set at 80% transmission at 800 mμ; white light excitation; slit widths some as above.

The delayed light emission spectrum for *Anacystis* shows no phycocyanin emission and is essentially the same as the *Chlorella* delayed light spectrum, except the peak is at 675 mμ (*Chlorella* 685 mμ). The fluorescence emission spectrum shows the phycocyanin emission between 620 and 660 mμ as well as the chlorophyll emission when the cells are illuminated with blue exciting light.

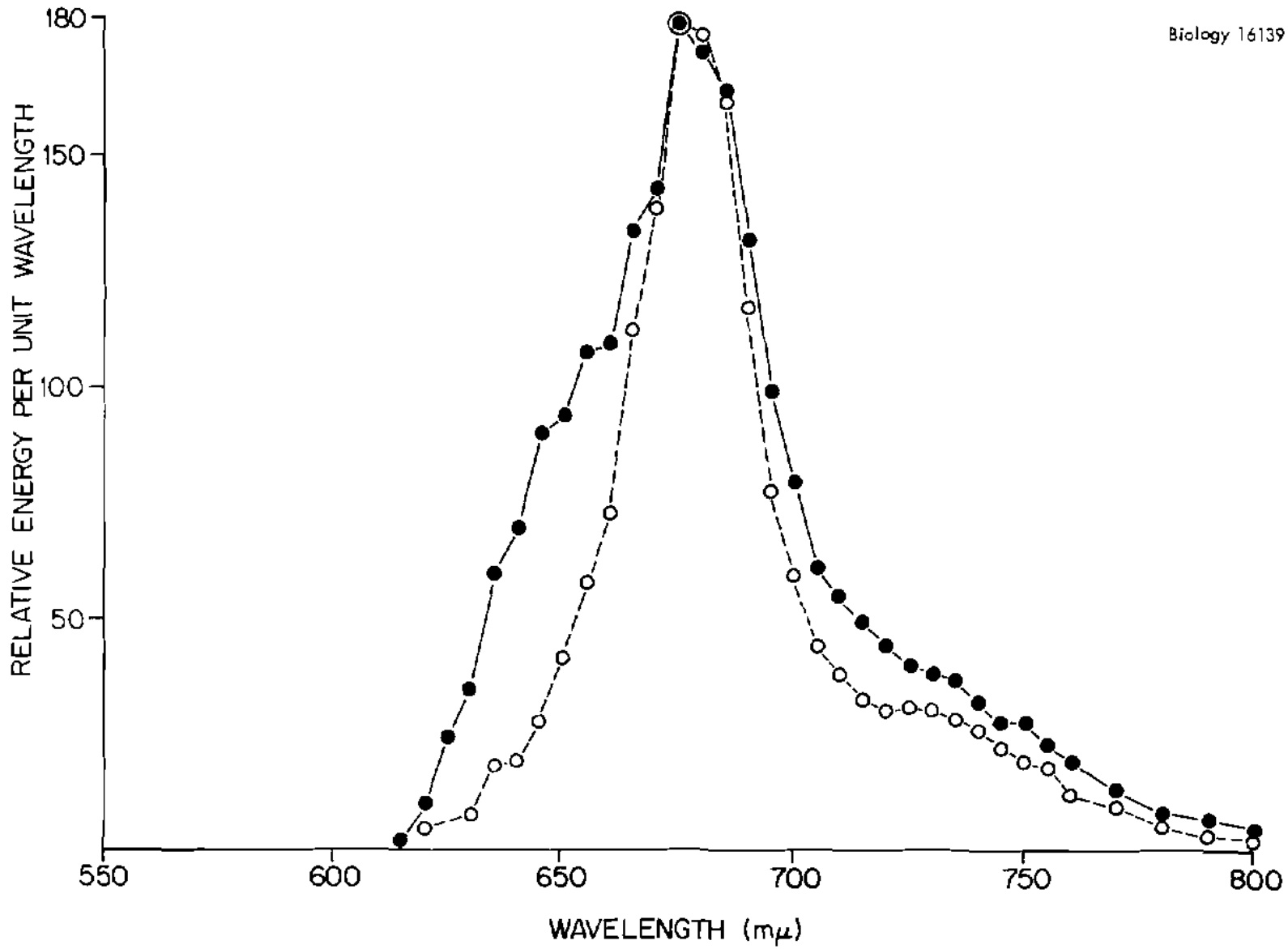

Biology 16139
RELATIVE ENERGY PER UNIT WAVELENGTH
180
150
100
50
550
600
650
700
750
800
WAVELENGTH (mμ)

Fig. 7. *Anacystis* Fluorescence Emission Spectrum (●—●). Cells 1 day old set at 80% transmission at 800 mμ; sodium light excitation with filter No. 9782 in exciting light beam; slit widths in millimeters (0.1 entrance, 0.17 exit). *Anacystis* delayed light emission spectrum (○--○). Same spectrum as in Fig. 6 except normalized to 0.5 scale in order to plot the delayed light spectrum under the sodium light fluorescence spectrum.

The fluorescence emission spectra show the phycocyanin emission much more intensified (peak at 645 mμ) when the cells were illuminated with sodium light than with blue light. If the chlorophyll emissions were subtracted from the spectrum, there would be phycocyanin emission at 645 mμ, a trough at 680 mμ, and extra phycocyanin emission between 700 and 750 mμ.

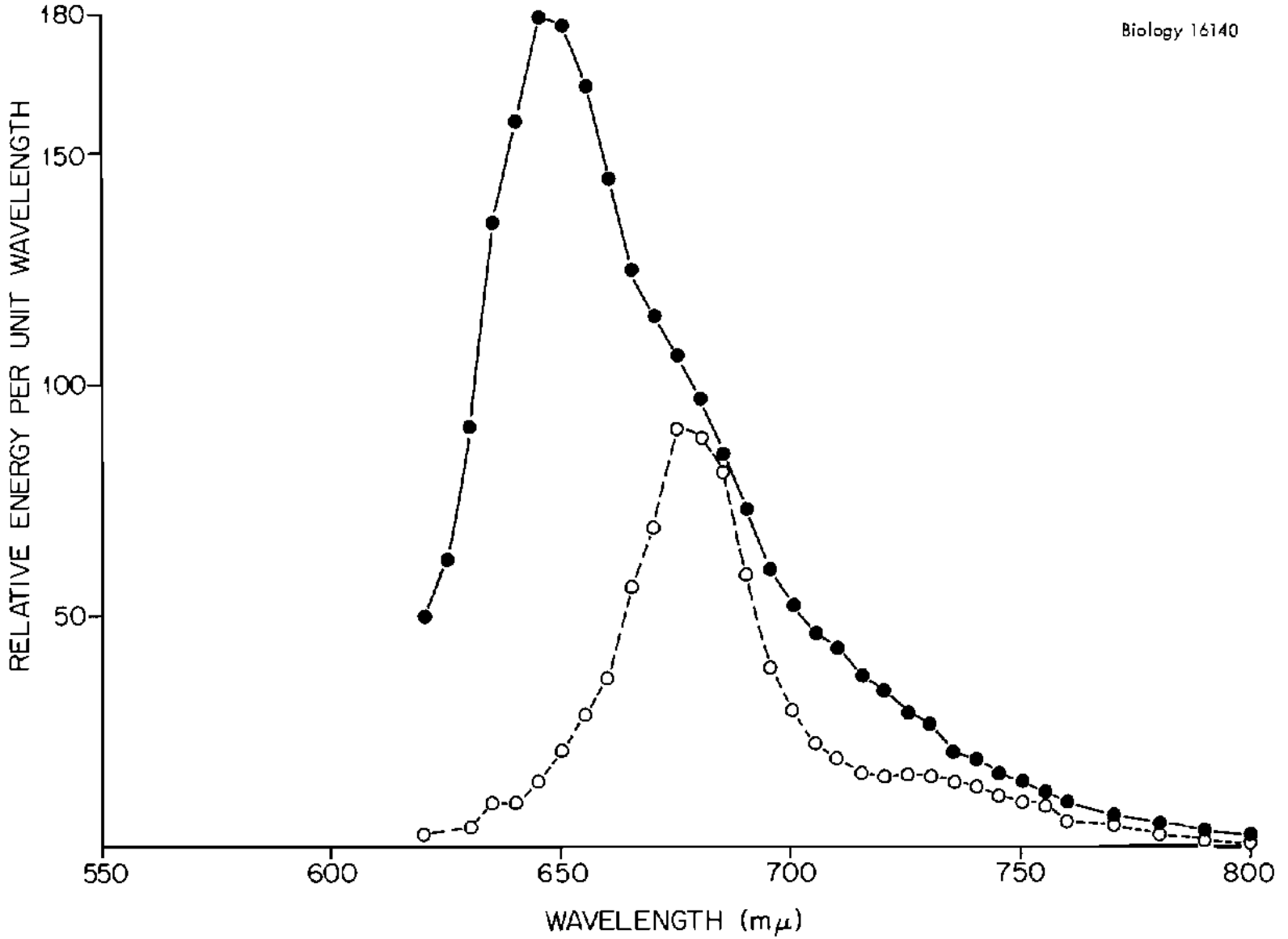

Biology 16140
RELATIVE ENERGY PER UNIT WAVELENGTH
180
150
100
50
550
600
650
700
750
800
WAVELENGTH (mμ)

Fig. 8. *Porphyridium* Fluorescence Emission Spectrum (●—●). Cells 5 days old set at a concentration of 7 $mm^3/10$ cc; blue light excitation; slit widths in millimeters (0.17 entrance, 0.24 exit). *Porphyridium* delayed light emission spectrum (○--○). Cells 6 days old set at a concentration of 7 $mm^3/10$ cc; white light excitation; slit widths in millimeters (0.2 entrance, 0.27 exit).

The delayed light emission spectrum was normalized underneath the fluorescence spectrum in order to keep the curves from overlapping. The fluorescence emission spectrum shows the emissions from phycoerythrin at 575 $m\mu$, phycocyanin at 655 $m\mu$, and chlorophyll at 675 $m\mu$. If the chlorophyll emission were subtracted from the spectrum, there would be phycoerythrin emission at 575 $m\mu$, phycocyanin emission at 655 $m\mu$, a trough at 675 $m\mu$, and extra phycacyanin emission between 700 and 750 $m\mu$.

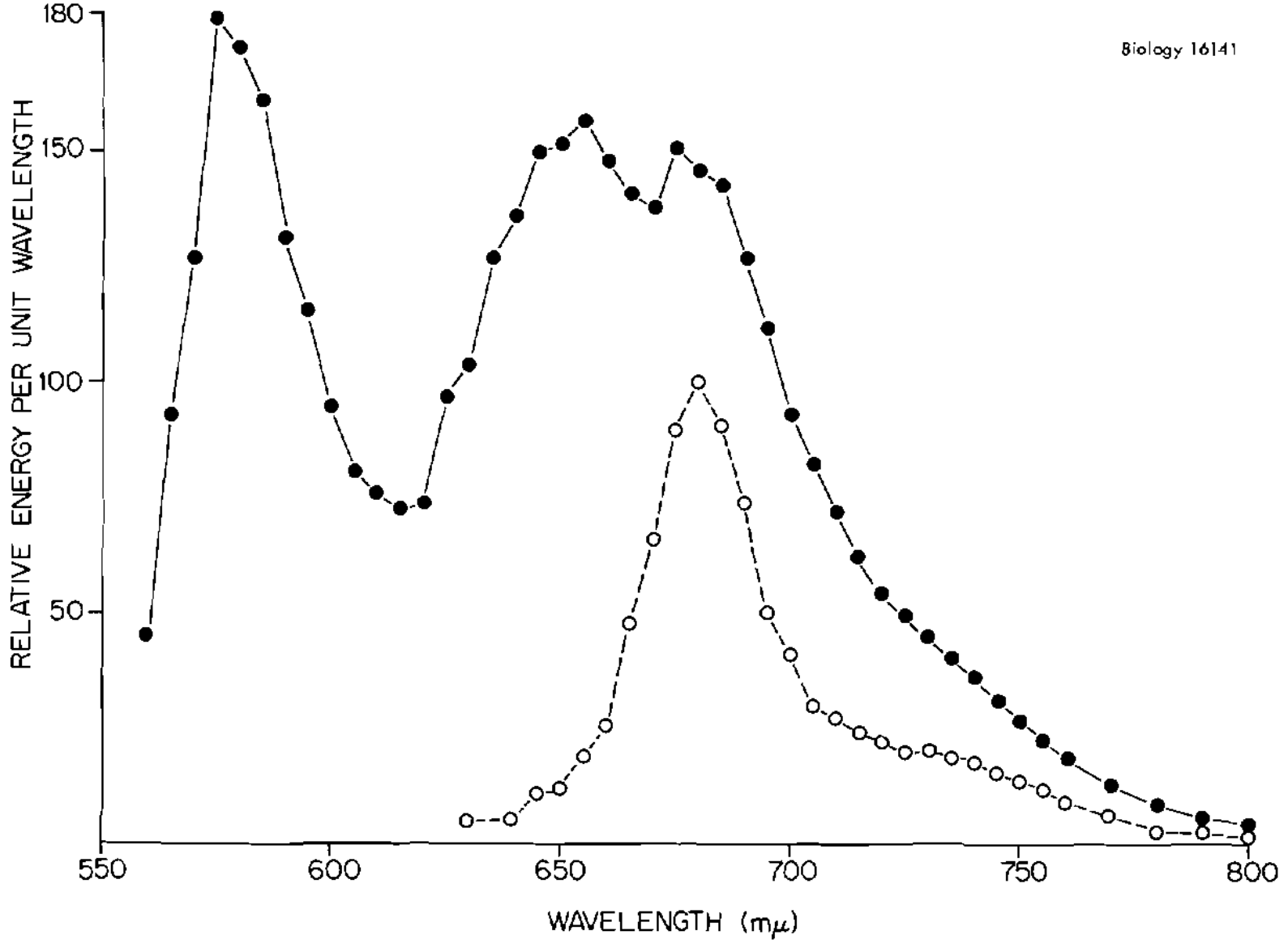

Biology 16141
RELATIVE ENERGY PER UNIT WAVELENGTH
180
150
100
50
550
600
650
700
750
800
WAVELENGTH (mμ)

Fig. 9. Summary of Three Delayed Light Emission Spectra Representing the Green, Blue-Green, and Red Algae.

The spectra show that the delayed light is coming from excited chlorophyll. None of our experiments showed any indication of delayed light emission from the accessory pigments.

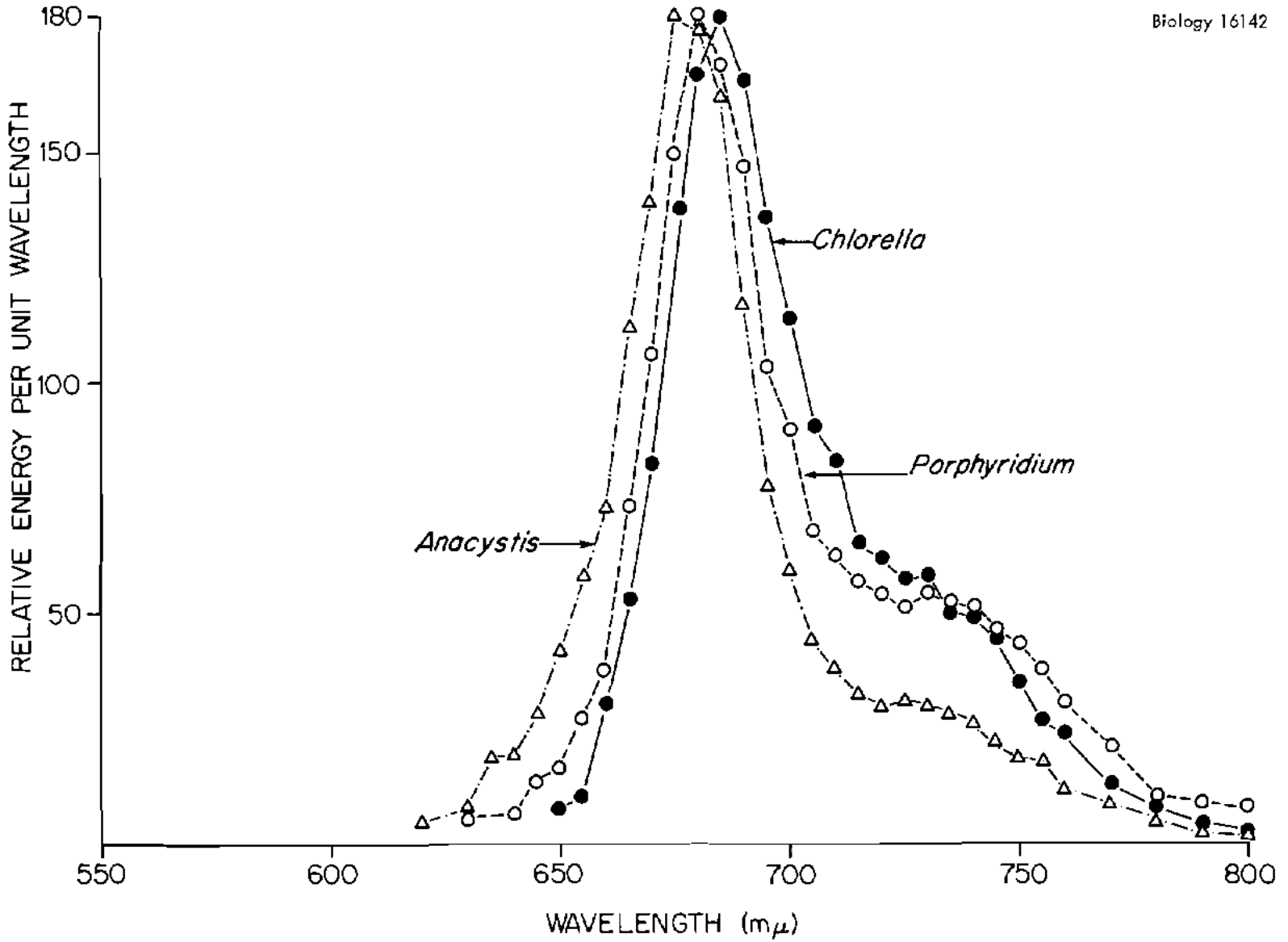

Biology 16142
RELATIVE ENERGY PER UNIT WAVELENGTH
180
150
100
50
550
600
650
700
750
800
WAVELENGTH (mμ)
Chlorella
Porphyridium
Anacystis

Fig. 10. ***Scenedesmus*** **Wild Type Fluorescence Emission Spectrum (●—●).** Cells 3 days old set at a concentration of 4.5 mm^3/10 cc; blue light excitation; slit widths in millimeters (0.1 entrance, 0.17 exit). *Scenedesmus* wild type delayed light emission spectrum (○--○). Cells 3 days old set at a concentration of 4.5 mm^3/10 cc; white light excitation; slit widths same as above.

The delayed light and fluorescence emission spectra for *Scenedesmus* wild type are similar to each other and to the two *Chlorella* spectra. The wavelength peaks are at 685 mμ.

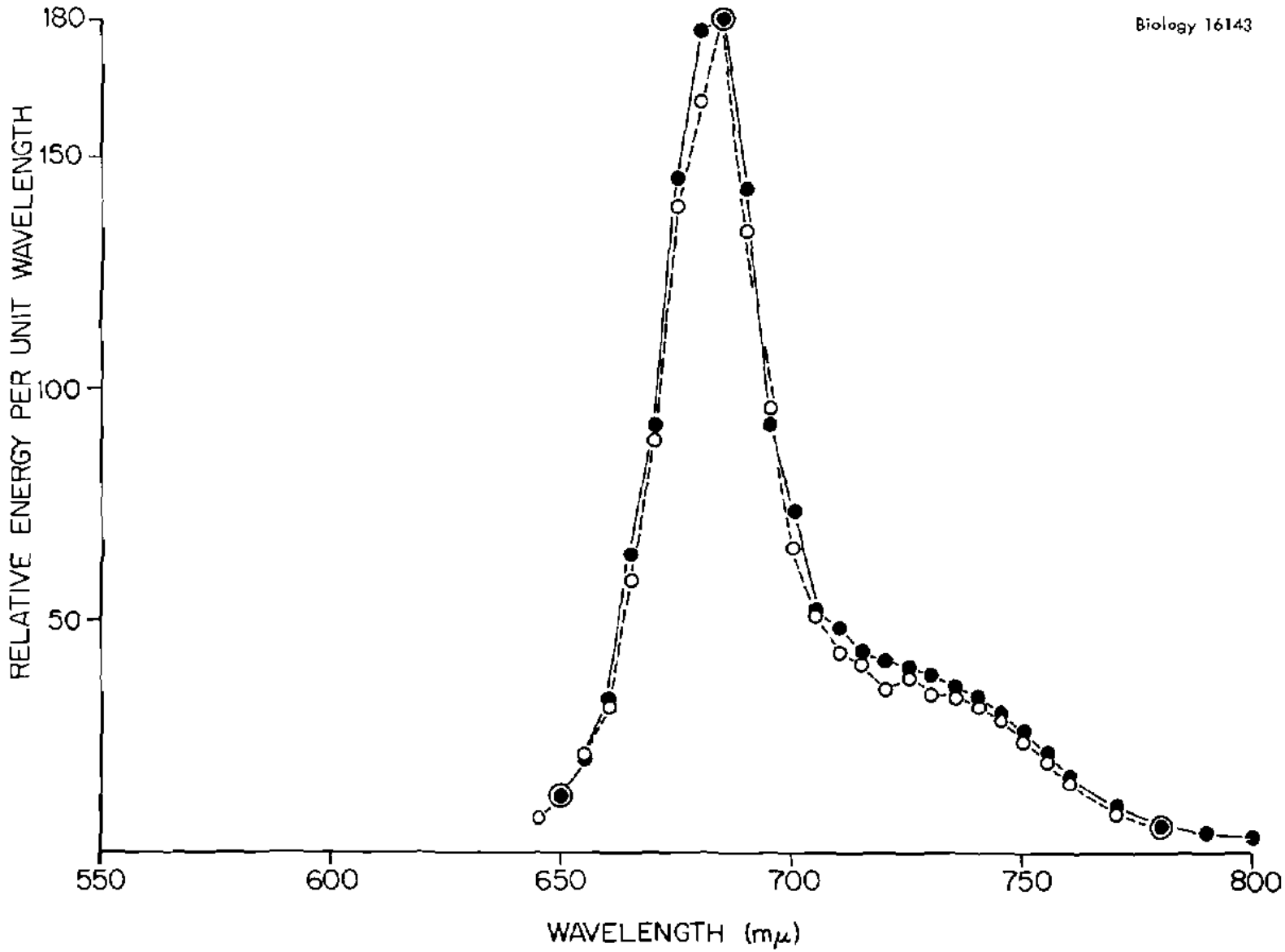

Biology 16143
RELATIVE ENERGY PER UNIT WAVELENGTH
180
150
100
50
550
600
650
700
750
800
WAVELENGTH (mμ)

Fig. 11. *Scenedesmus* Mutant 8 Fluorescence Emission Spectrum (●—●). Cells 2 days old set at a concentration of 3.5 $mm^3/10$ cc; blue light excitation; slit widths in millimeters (0.1 entrance, 0.17 exit). *Scenedesmus* mutant 8 delayed light emission spectrum (o---o). Cells 3 days old set at a concentration of 5 $mm^3/10$ cc; white light excitation; slit widths same as above.

The delayed light emission spectrum for mutant 8 is similar to the delayed light spectra of *Chlorella* and the wild type. The fluorescence emission spectrum, however, shows a higher fluorescence emission out in the long-wavelength region as compared with the wild type and mutant 11, yet it does not have P-700.[9,10]

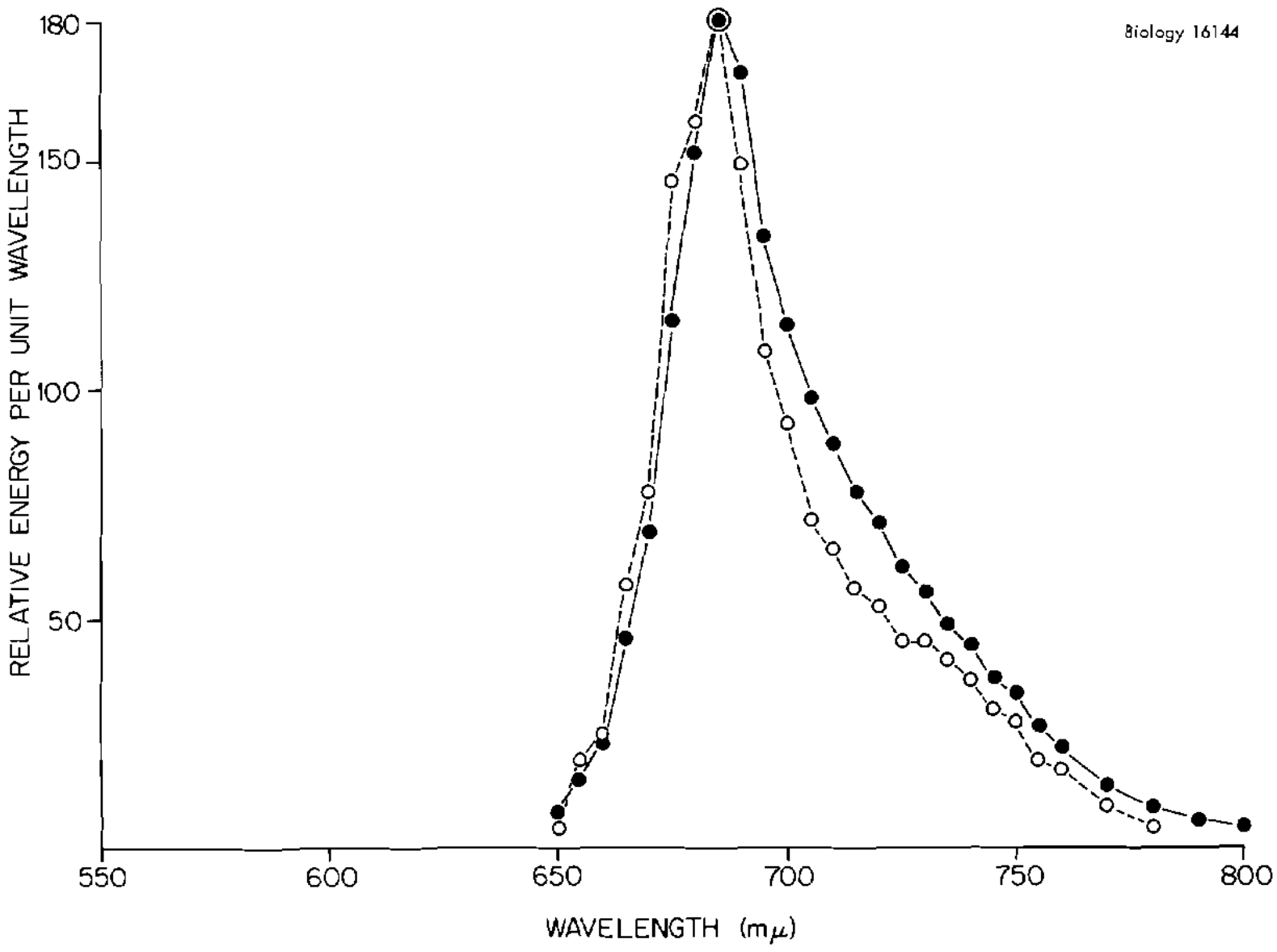
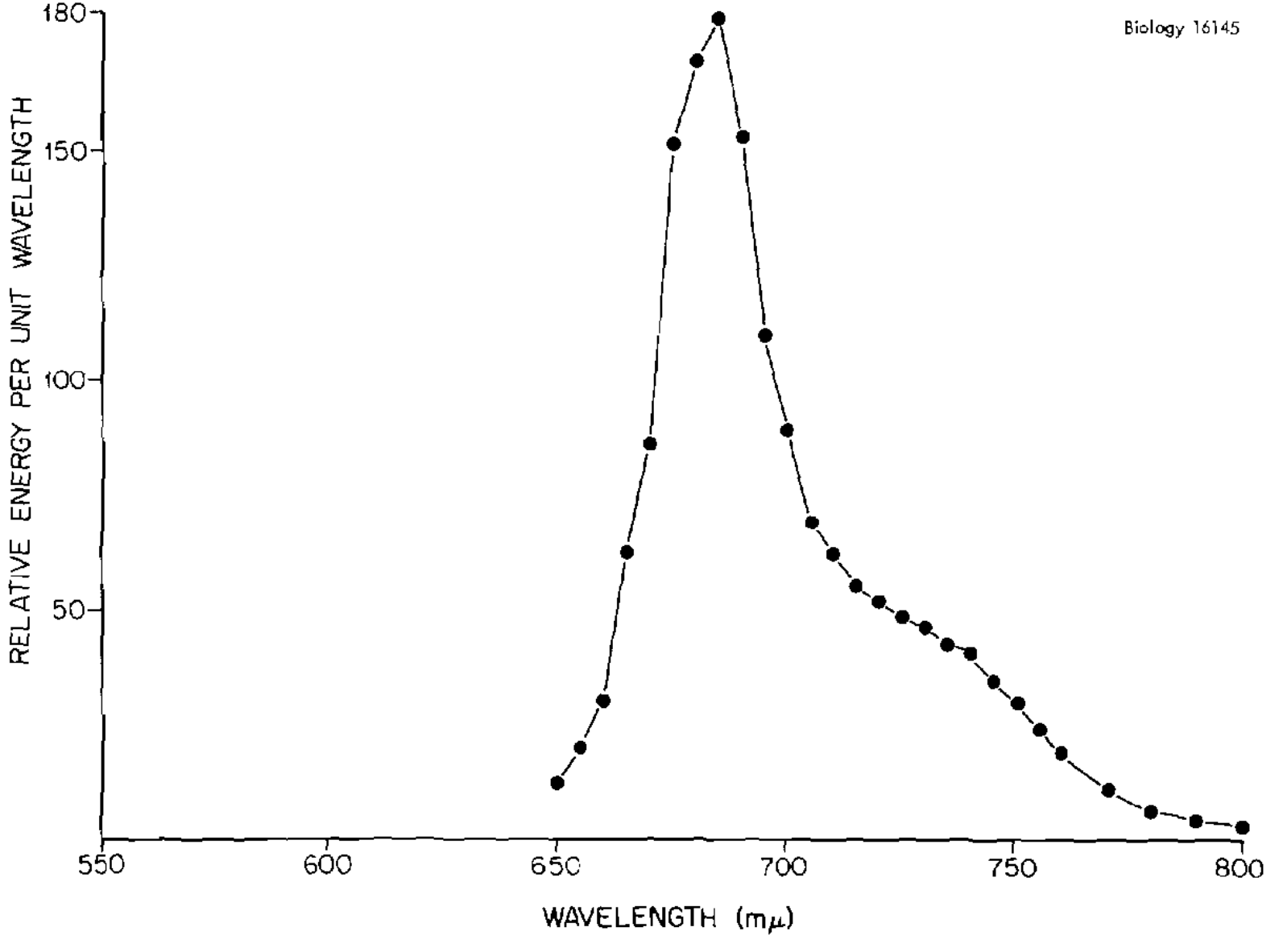

Biology 16144
RELATIVE ENERGY PER UNIT WAVELENGTH
180
150
100
50
550
600
650
700
750
800
WAVELENGTH (mμ)

Fig. 12. *Scenedesmus* Mutant 11 Fluorescence Emission Spectrum (●—●). Cells 3 days old set at a concentration of 4.5 $mm^3/10$ cc; blue light excitation; slit widths in millimeters (0.1 entrance, 0.17 exit).

The delayed light intensity from mutant 11 is too weak to allow a delayed light emission spectrum to be made with our equipment. The fluorescence emission spectrum for mutant 11 is the same as those of *Chlorella* and *Scenedesmus* wild type with the wavelength peak at 685 $m\mu$.


Biology 16145
RELATIVE ENERGY PER UNIT WAVELENGTH
180
150
100
50
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600
650
700
750
800
WAVELENGTH (mμ)

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