# ANNUAL RESEARCH REPORT

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Jerome J. Wolken, Principal Investigator
Biophysical Research Laboratory
Carnegie Institute of Technology
Pittsburgh, Pennsylvania 15213

NGR-39-002-011

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## RESEARCH PROGRESS

Instrumentation research during the past year has been directed towards the design, construction, and application of specialized instrumental devices and technics for the study of cells--in the living state. Particular emphasis was directed towards the identification of pigment molecules, e.g. porphyrins, chlorophylls, hemoglobins, carotenoids. These pigments are directly related to the energetics of living cells; and therefore, the ability to identify and follow their synthesis has much to tell us about these kinds of organic molecules and the life processes.

### Microspectrophotometer

A microanalytical method for the study of the spectroscopy of the cell (e.g. algae, animal tissue cells, and red blood cells) and its organelles (e.g. chromatophores, chloroplasts, nucleus) is microspectrophotometry. The instrumentation to achieve this was begun in our laboratory in 1960, and there are now variously designed microspectrophotometers designated as M-1, M-2, M-3, and M-4 in our laboratory. The design, performance, and application of these instruments to the study of pigments in a variety of cells have been presented in various published reports. Because of limitations in these instruments, a completely new instrument, M-5, has been under development in our laboratory for the past two years. The limitations of the microspectrophotometers M-3 and M-4 are their response time,

which is limited by the electronics and the recorder, and focusing; the latter being due to the optics and design of the microscope.

A schematic of the microspectrophotometer M-5 is illustrated in Figure 1, and a photograph of the instrument is shown in Figure 2. The adaptability of this instrument to space exploration has been considered in its design, as well as its feasibility for better specimen handling and focusing.

## Component Parts of Microspectrophotometer M-5

Microscope. The instrument uses an American Optical microscope base. The optics are Zeiss ultrafluar objectives, which require a special eyepiece. Less vibration was encountered with the use of a Unitron BTMS microscope stage; and moreover, the stage was easily adapted to the microscope.

In order to view specimens which are photosensitive, additional equipment is necessary; a cold stage on the microscope helps to reduce thermal "bleaching" effects, and an infrared image converter is also used. In order to overcome inadequacies for specimen handling, an image intensifier tube is being investigated which allows us to view specimens under very low light levels of illumination and permits increased optical resolution.

Detector. The detector is an EMI 9558Q Photomultiplier with a housing to cool the photomultiplier. The photomultiplier enables the instrument to make measurements with lower levels of light and improves the instrument sensitivity. The EMI 9558QA Photomultiplier Tube has a spectral response from 162 mμ to 840 mμ, with a quantum

efficiency of about 20 per cent from 200 to 420 mμ, 10 per cent efficiency at 480 mμ, and 1 per cent efficiency at 785 mμ. The dark current noise is extremely low, and with cooling (-12°C.) is almost negligible.

Monochromator and light source. The monochromator used is a Canalco rapid-scanning one, which has 600 line/mm. grating and has ten different speeds, from two seconds to 1000 seconds, over a 200 to 800 mμ range. Its dispersion is 4 mμ/mm. and peaks at 500 mμ. A disadvantage of all grating monochromators is that the light is about 10 to 20 per cent polarized. (A part of this polarization is removed by using a quartz diffusing plate.)

A quartz lens of about a 10 cm. focal length was inserted into the light path in front of the aperture and adjusted so that the filament in the source and the slit was out of focus. This was found to concentrate more of the light on the aperture.

Chopper. The accuracy in measuring the relative percentage of absorption (as far as electronics are concerned) depends on the relative heights of the two pulses; the sample and reference areas are not measured simultaneously but alternately in time. Therefore, if the overall accuracy is to stay within one per cent, any change in the relative height of the pulses due to relative spectral quantum efficiency of the photocathode, the relative spectral distribution of the light source, or the gain of the amplification should be within one per cent during the time for one pair of pulses. Consequently, a short-scanning time requires a correspondingly short pulse pair-time. The desired short pulse pair-time required the development of a precision, high-speed chopper and special electronic circuits in

the amplifier. The chopper in the M-5 has been the main design improvement over previous instruments. (See Figures 3 and 4)

Amplifier. The electronics consist of a preamplifier mounted on the case of the photomultiplier tube, a main amplifier, an electronic switch, an automatic gain amplifier, a feedback circuit, an integrating circuit for each sample and reference pulse, and a panel for necessary controls. (Figure 5)

The lead from the photomultiplier to the preamplifier is as short as possible in order to minimize lead-in capacity. The preamplifier then has a current gain of about -150X. The main amplifier gives the final amplification to the signal, which at this point consists of both sample and reference pulses alternately in time. The electronic switch, synchronized to the chopper, then separates the signal. The reference pulse goes to the automatic gain amplifier, where it is further amplified, and then to the feedback circuit.

The feedback circuit consists of a field effect transistor which acts as the load resistance for the photomultiplier. The output from the automatic gain amplifier is connected to the gate of the field effect transistor. The resistance of the field effect transistor depends on the potential on its gate in such a way that low voltage causes a low resistance, and high voltage causes a high resistance. As the signal from the photomultiplier increases, the output of the automatic gain amplifier, which goes to the gate of the field effect transistor, decreases. There is also a corresponding decrease in load resistance which decreases the signal.

The circuit holds the reference pulse constant over a range of 250 times within two per cent. Now, the sample pulse is simply integrated and sent to the recorder where the data is plotted.

Recorder. The recorder for the M-5 is a D'Arsonval-type pen drive, rather than a servomechanism-type recorder used for the M-3 and M-4 instruments. Also, the electronics are much more versatile and enhance the response speeds.

The new microspectrophotometer, M-5, will scan the visible spectrum, 400 to 700 mμ in less than 10 seconds or 100 wave numbers in 0.33 seconds. (See, for example, studies of RNA incorporated into Plate VIII, proteinoid microspheres.)

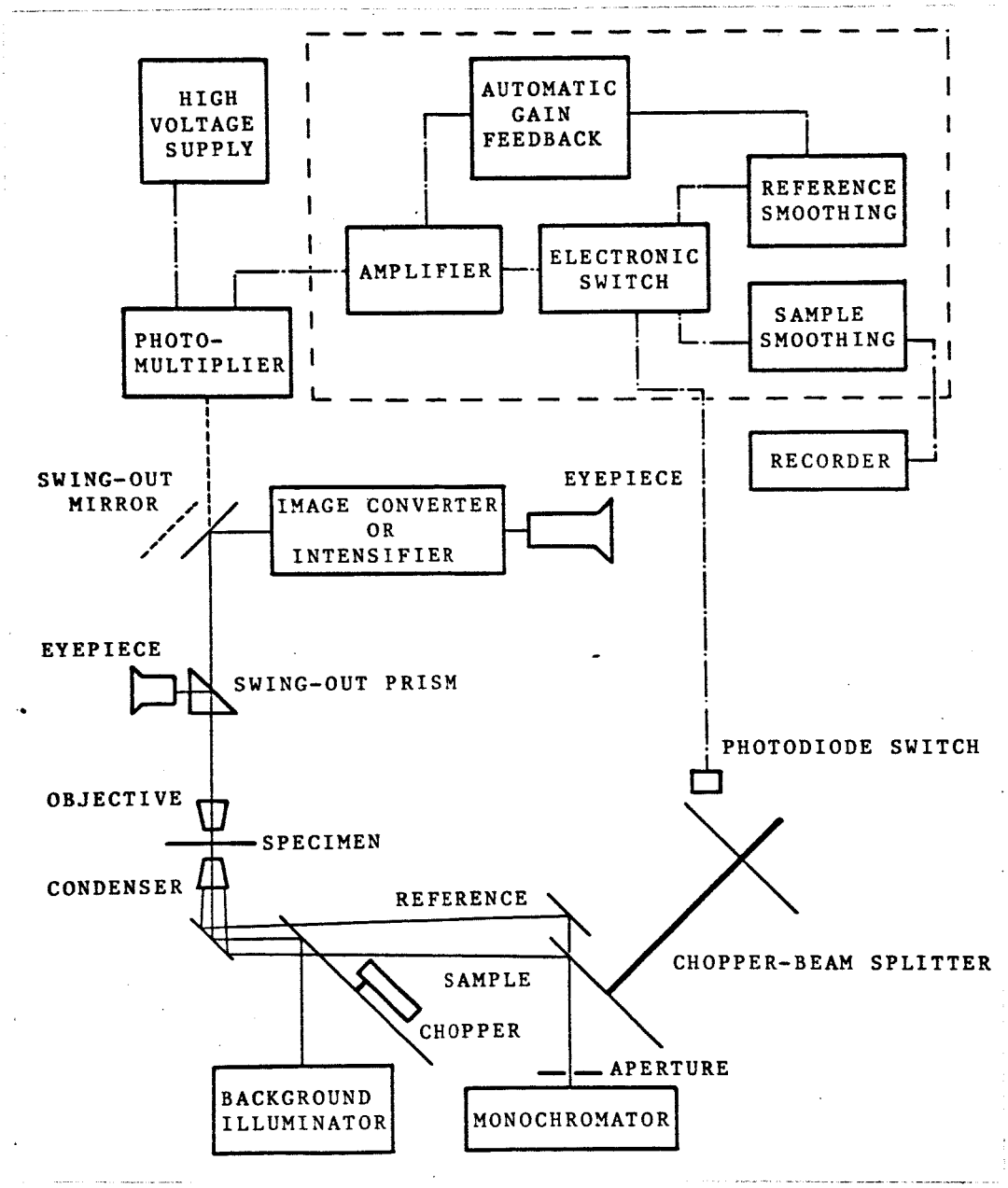



FIGURE 1 Block diagram of the M-5.

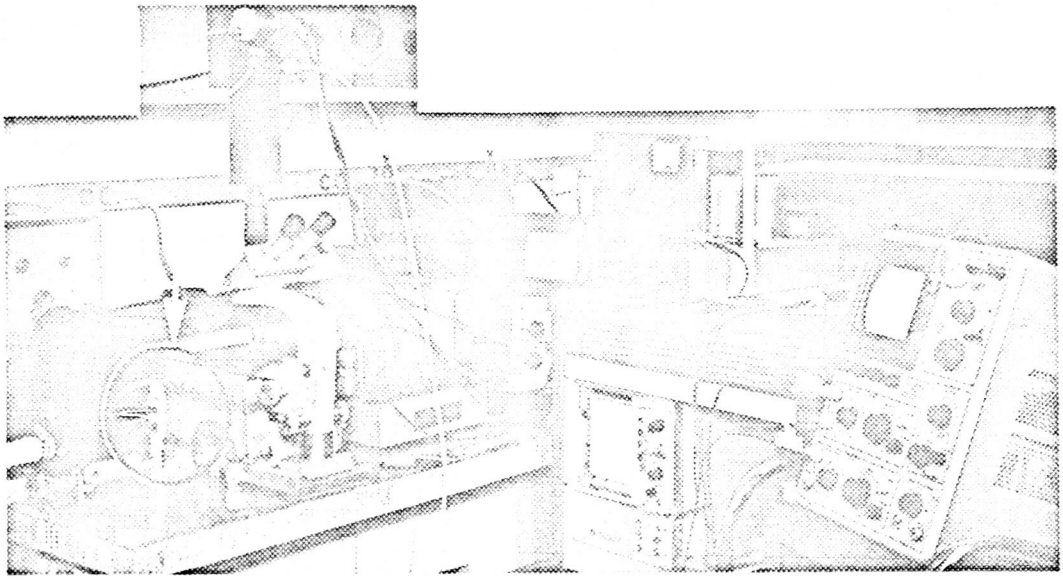


FIGURE 2 Microspectrophotometer, M-5.

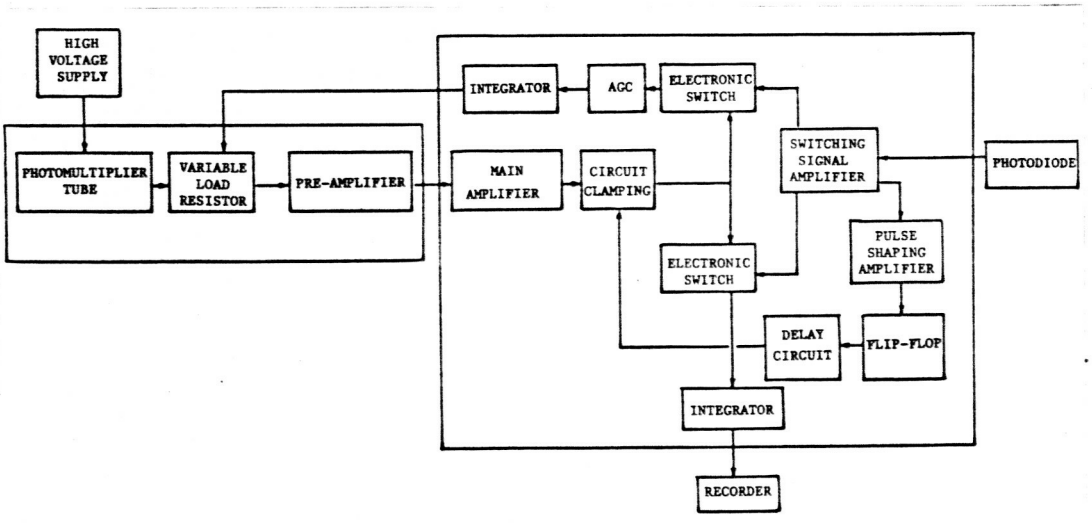
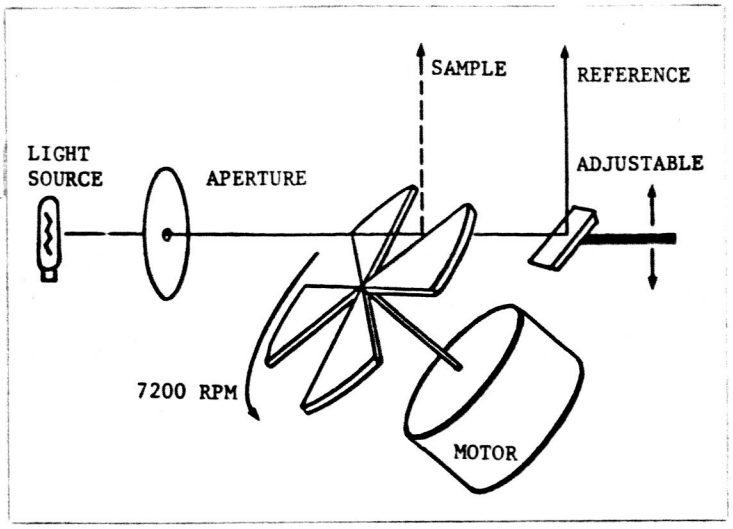



FIGURE 3 Electronic schematic of M-5.

FIGURE 4

Schematic diagram of chopper.



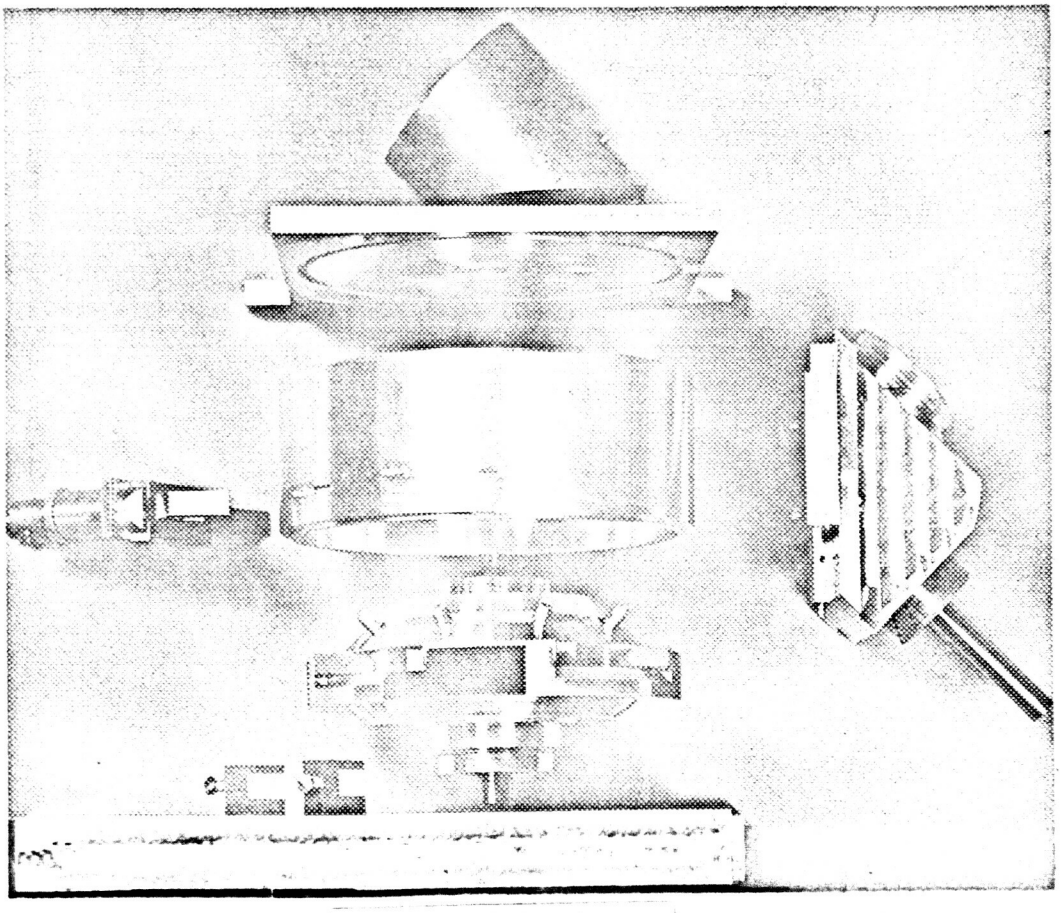



FIGURE 5 Chopper.

RESEARCH STAFF SUPPORTED WHOLLY OR IN PART FROM NASA GRANT NGR-39-002-011

| | |
|---|---|
| Mahendra Singh | B.S., M.S., Agra University<br>Ph.D., University of Florida<br>Research Biologist |
| Robert G. Florida | B.S., University of Pittsburgh<br>Research Biologist |
| Robert D. Forsberg | B.S., Westminster College<br>Research Biologist |
| Wendy E. Beck | B.A., Allegheny College<br>Secretary |
| Georgianna G. Moore | B.S., Carnegie Institute of Technology; Secretarial Assistant |
| Barbara A. Runnette | Technical Assistant |

GRADUATE STUDENTS SUPPORTED IN PART

| | |
|---|---|
| Barbara Fagan | B.S., Biology, Carnegie Institute of Technology |
| Daniel Strittmater | B.S., La Crosse State University<br>M.S., Mathematics, Carnegie Institute of Technology |

SUMMER STUDENT ASSISTANTS SUPPORTED

| | |
|---|---|
| Michelle Bennet-Alder | Chemistry, Michigan State University |
| Erich Knobil | Physics, Cornell University |

## RELATED PUBLICATIONS 1965-1966

Wolken, J. J. Vision: Biophysics and Biochemistry of Retinal Photoreceptors, Charles C. Thomas, Springfield, Illinois, 1966.

Wolken, J. J., "Photoreceptor Structures and Energy Transfer," J. Arkansas Medical Society, 62: 61, 1965.

Wolken, J. J., "Molecular and Fine Structure of Photoreceptors," in Recent Progress in Photobiology, edited by E. J. Bowen, Academic Press, London, England, 145-151, 1965.

Wolken, J. J., "Microspectrophotometry," in Encyclopedia of Chemistry, edited by G. L. Clark, Reinhold Publishing Company, New York, 1965.

Marak, G. E. and J. J. Wolken, "An Action Spectrum for the Fire Ant: Solenopsis saevissima," Nature, 205: 1328, 1965.

Wolken, J. J. and G. J. Gallik, "The Compound Eye of a Crustacean: Leptodora kindtii," J. Cell Biology, 26: 968, 1965.

Wolken, J. J., "The Chloroplast," in The Growth and Development of the Chloroplast to Photosynthesis, edited by C. Sironval, Edition Masson, Paris (in press).

Wolken, J. J., "Lipids and the Molecular Structure of Photoreceptors," in The Biochemistry of Lipids, edited by F. A. Kummerow (in press).

## RELATED REPORTS OF RESEARCH TO SCIENTIFIC MEETINGS 1965-1966

Wolken, J. J., September 13-18, 1965. "The Chloroplast in Photosynthesis," symposium on "Growth and Development of the Chloroplasts to Photosynthesis," Gorsem, Belgium.

Wolken, J. J., October 11-13, 1965. "Lipids and the Molecular Structure of Photoreceptors," Lipid Biochemistry Symposium, American Oil Chemists' Society, Cincinnati, Ohio.

Zdrojkowski, R. J. and R. D. Forsberg, November 10-12, 1965. "A Low Light Level Microspectrophotometer," 18th Annual Conference on Engineering in Medicine and Biology, Philadelphia, Pennsylvania.

Wolken, J. J. and G. J. Gallik, February 23-26, 1966. "Studies of Isolated Intact Frog Retinal Rods," Biophysical Society Meetings, Boston, Massachusetts.

CHARACTERISTICS OF MICROSPECTROPHOTOMETERS

M-1
a. Manual
b. Clairex 103 CL photoconductive sensor
c. Oscilloscope read out
d. Spectral range 240 mμ to 1200 mμ

M-2
a. Automatic
b. Clairex 103 CL photoconductive sensor
c. Brown recorder read out
d. Spectral range 240 mμ to 1200 mμ
e. 400 to 700 mμ scan in 5 to 10 minutes

M-3
a. Improved automatic M-2
b. Clairex 103 CL photoconductive sensor
c. Varian recorder read out
d. Spectral range 240 mμ to 1200 mμ
e. 400 to 700 mμ scan of the order of 5 minutes

M-4
a. Same as M-3
b. Photomultiplier sensor EMI type 9558B
c. Varian recorder read out
d. 400 to 700 mμ scan of the order of 5 minutes

M-5
a. Completely new electronically and mechanically
b. Photomultiplier sensor EMI type 9558B
c. Recorder read out (Sanborn Model 7700)
d. 400 to 700 mμ scan of the order of 5 to 10 seconds

## APPLICATIONS

### PLATE I

1. Freshly isolated retina from the eye of a frog, a single retinal rod outer segment. X1200

2. Spectra of frog rod outer segment; a, taken immediately upon isolation, spectrum indicates the visual pigment rhodopsin; spectra b, c, and d taken after bleaching with white light. Note shift in spectra d from rhodopsin to retinene (vitamin $A_1$ aldehyde). (Using microspectrophotometer M-4 on an area of 12 $\mu^2$.)

3. Purified extract of frog retinal rod outer segments (in 1 per cent digitonin); spectra obtained with Cary-14 spectrophotometer. Compare this data to Illustration 2 above.

### PLATE II

1. Eyespot granules of Euglena gracilis. X26,000

2. Spectrum of eyespot of Euglena (streptomycin) mutant. (M-4)

3. Spectrum of eyespot of Euglena, photosynthetic wild-type Z strain. (M-4)

The pigments of the eyespot have not been identified, although there is a similarity to carotenoids, e.g. β-carotene.

4. Eye of crustacean, Leptodora kindtii. X100

5. Spectrum of eye facets; note similarity to Plate 1, 2a. (M-3)

### PLATE III

1. Colored, pigmented, oil globules in wood turtle (Clemmys insculpa) eye. X575

3. Spectra of these colored globules. (M-4)

From such data, one can see how these globules behave as cut-off filters and are also important for any theories regarding color vision.

PLATE IV

1. Spectrum of yellow oil globules in retinal cone inner segment of frog eye. (M-4)

2. Spectrum of pigment granules in compound eye of crustacean, Daphnia (water flea). (M-4)

PLATE V

1. Spectrum of chlorophyll in the chloroplast, identified as almost all chlorophyll a. (M-4)

   a, chloroplasts of Euglena gracilis. X6500

2. Ultraviolet spectrum of chloroplast; note absorption peaks characteristic of protein and lipids. (M-1)

3. Spectrum of dark-grown Euglena cell; although it no longer contains chlorophyll, it does contain carotenoids. (M-4)

PLATE VI

1. Biosynthesis of chlorophylls and carotenoids (in Euglena gracilis) followed with time in the light. (M-4)

2. The bleaching and degradation of pigments, e.g. chlorophyll; with time in darkness. (M-4)

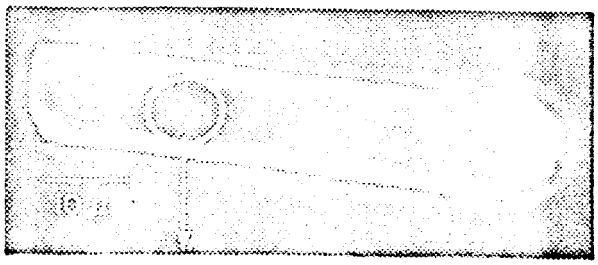
PLATE VII Red blood cell of frog. Note the spectral detail for hemoglobin. (M-4)

PLATE VIII

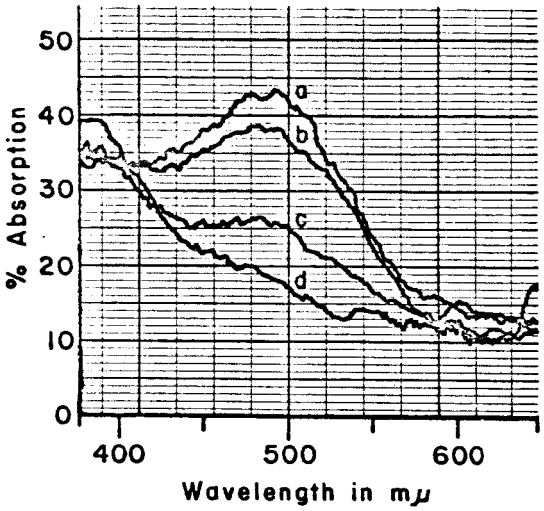
1. Proteinoid microspheres. Made from polymerization of amino acids (according to Fox) in which RNA (ribonucleic acid) has been incorporated. X2400

2. Spectrum of stained microspheres (Allied Chemical methyl green pyronin Y, a specific stain for RNA). Note that this spectrum was obtained in 8 seconds. (M-4)

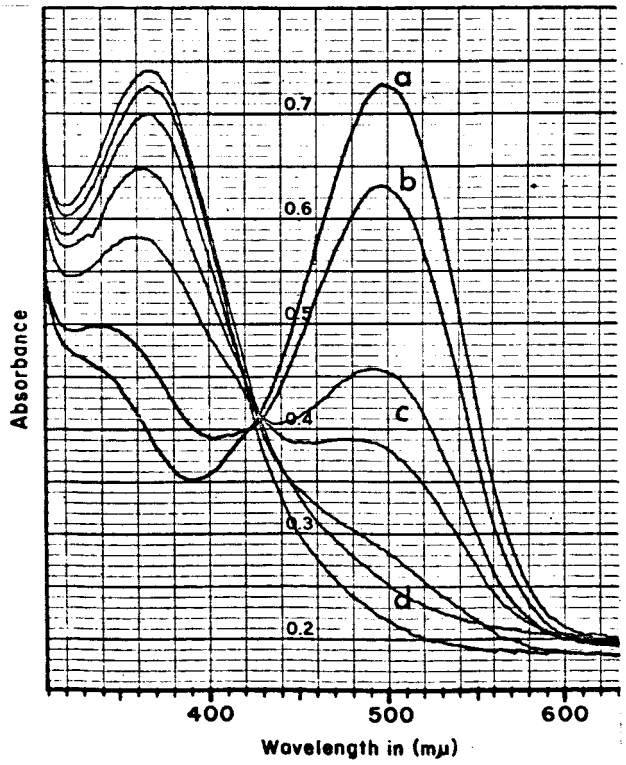
PLATE IX Spectra of chlorophyll in the Euglena chloroplast recorded with the M-5 instrument. Both spectra are of the same chloroplast in a live cell. Compare these spectra, which took ten seconds each, with Plate V,1 which took five minutes to record.



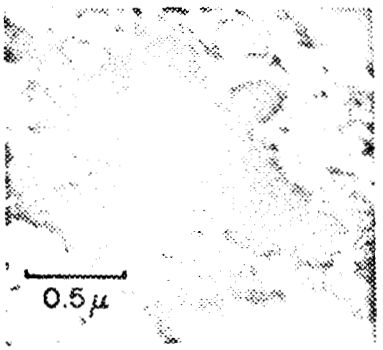
1. Frog retinal rod.

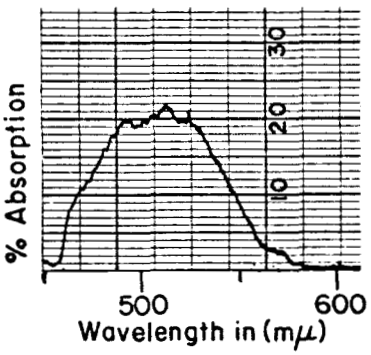


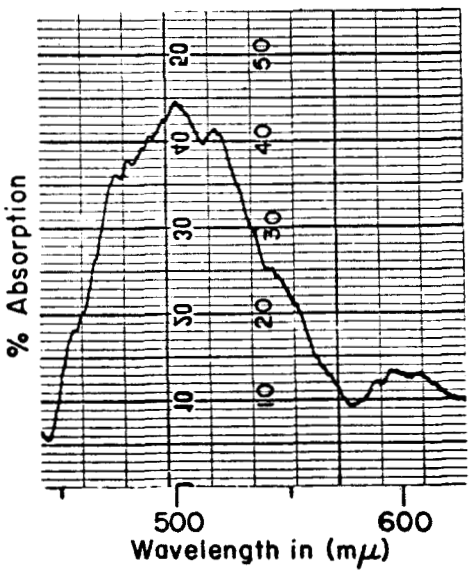

2. Spectra of frog retinal rod.




3. Spectra of an extract from frog retinal rods - rhodopsin.



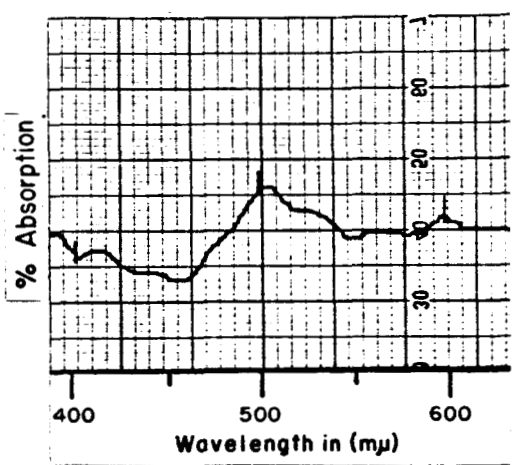




1. Euglena eyespot granules.

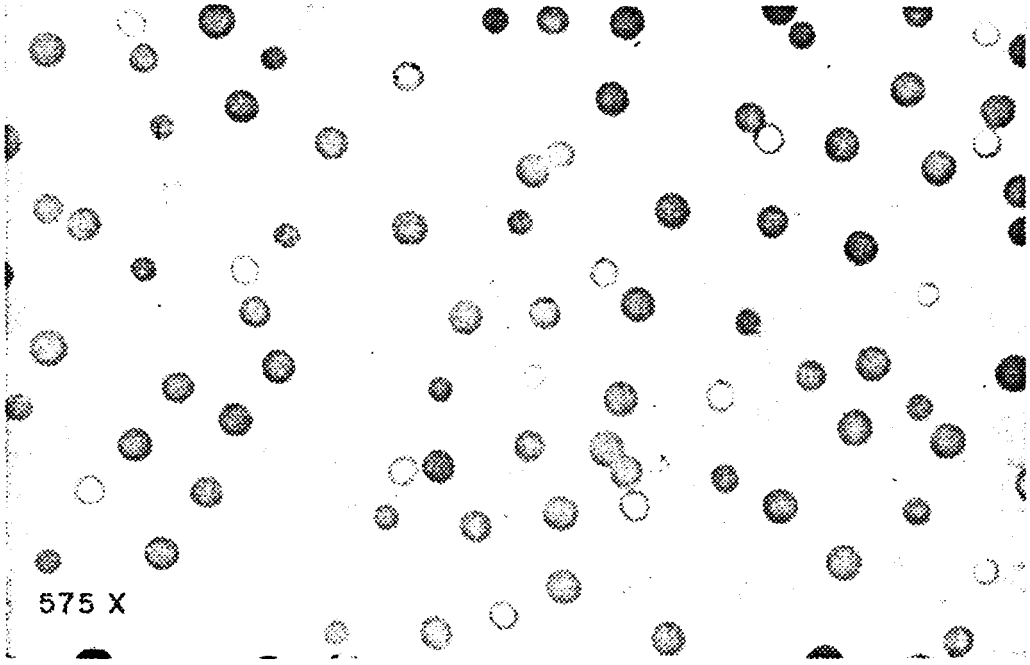
2. Spectrum of eyespot.

3. Spectrum of eyespot.

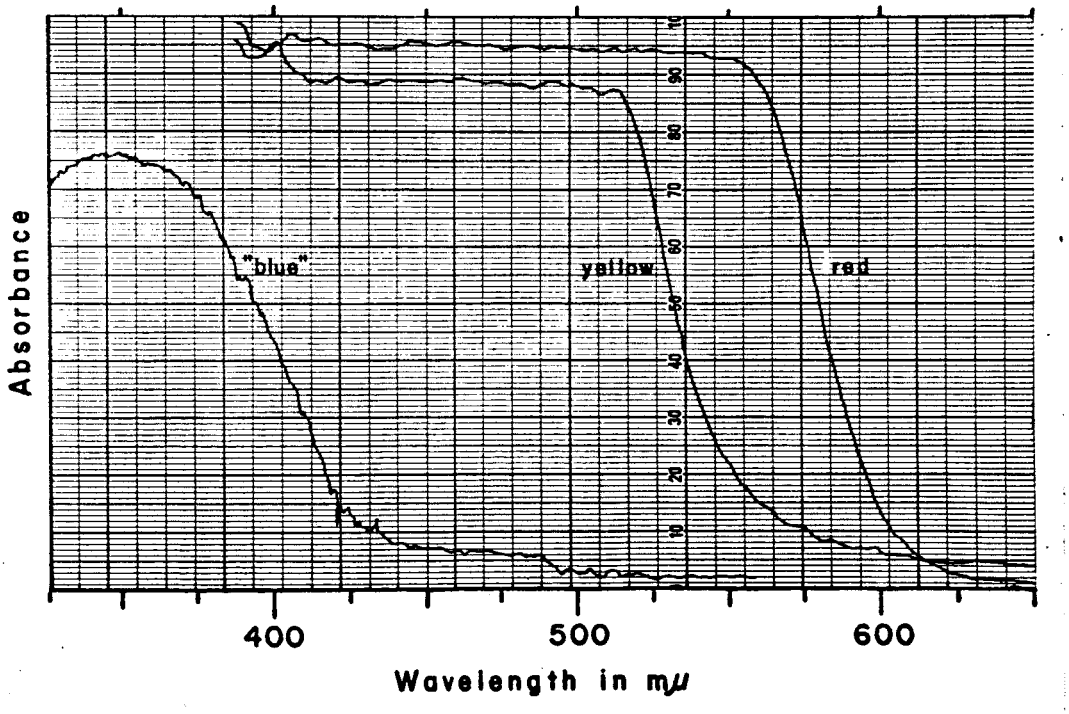
4. Leptodora compound eye.



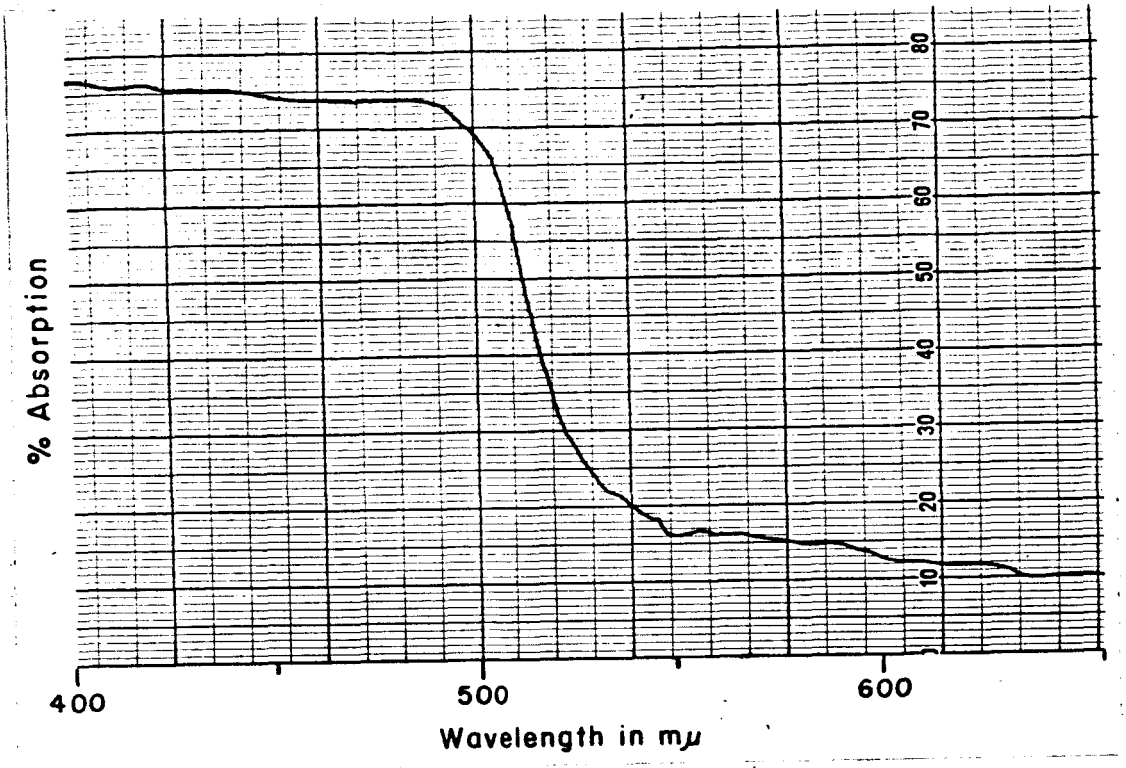

5. Spectrum of eye facets.



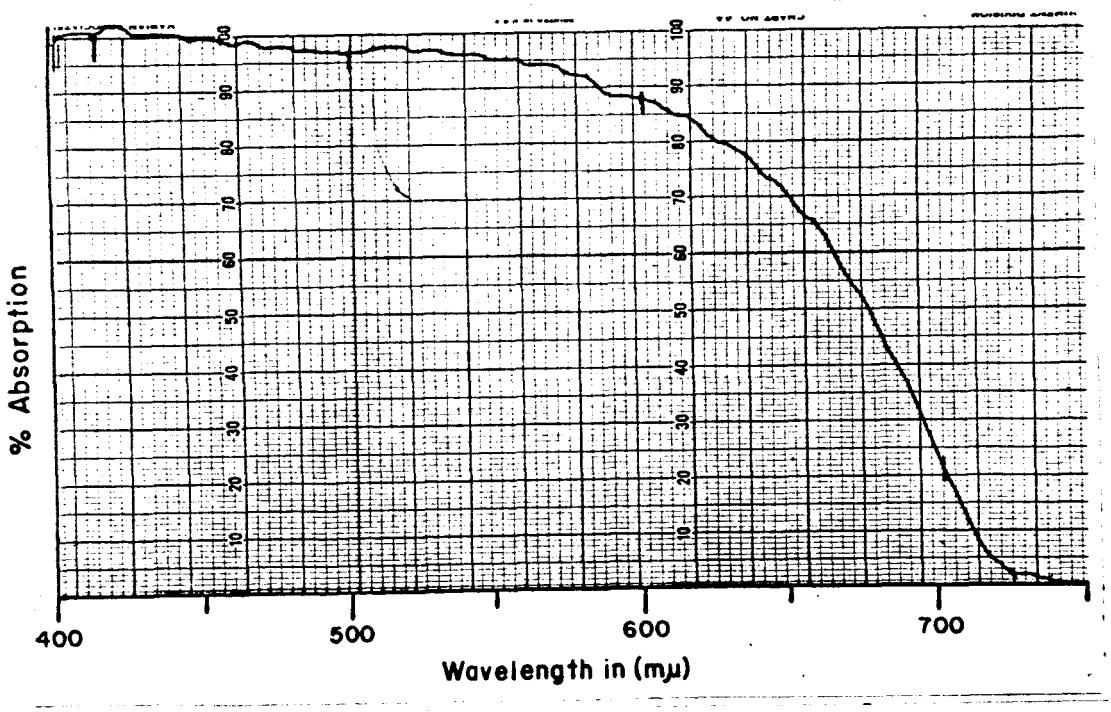

1. Colored oil globules in the retina of the wood turtle.



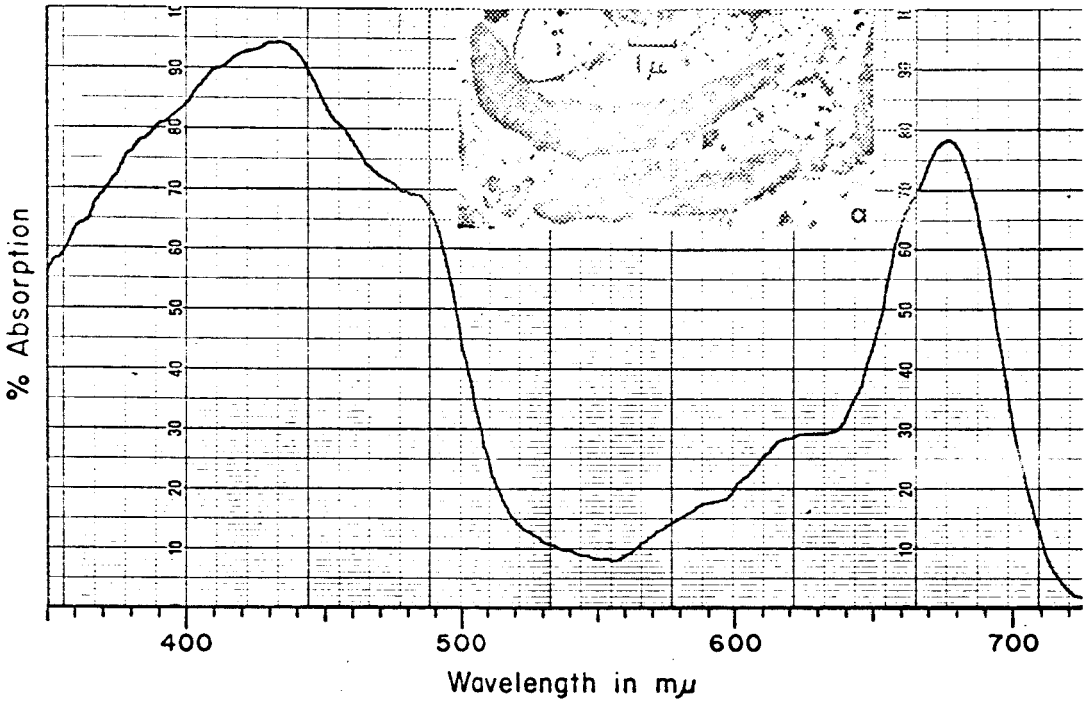

2. Spectra of the oil globules.



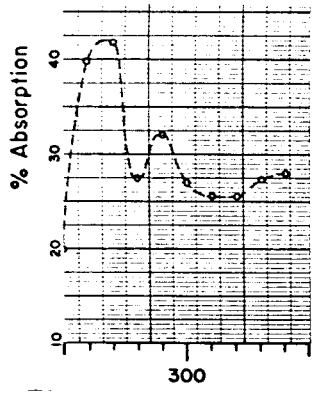

1. Spectrum of the yellow oil globule in the retina of the frog eye.




2. Spectrum of the pigment granules in the eye of Daphnia.

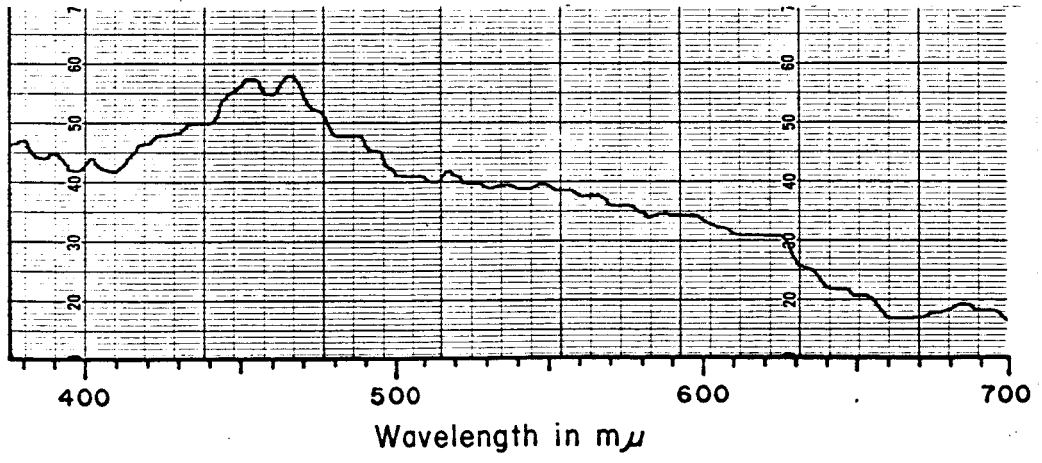


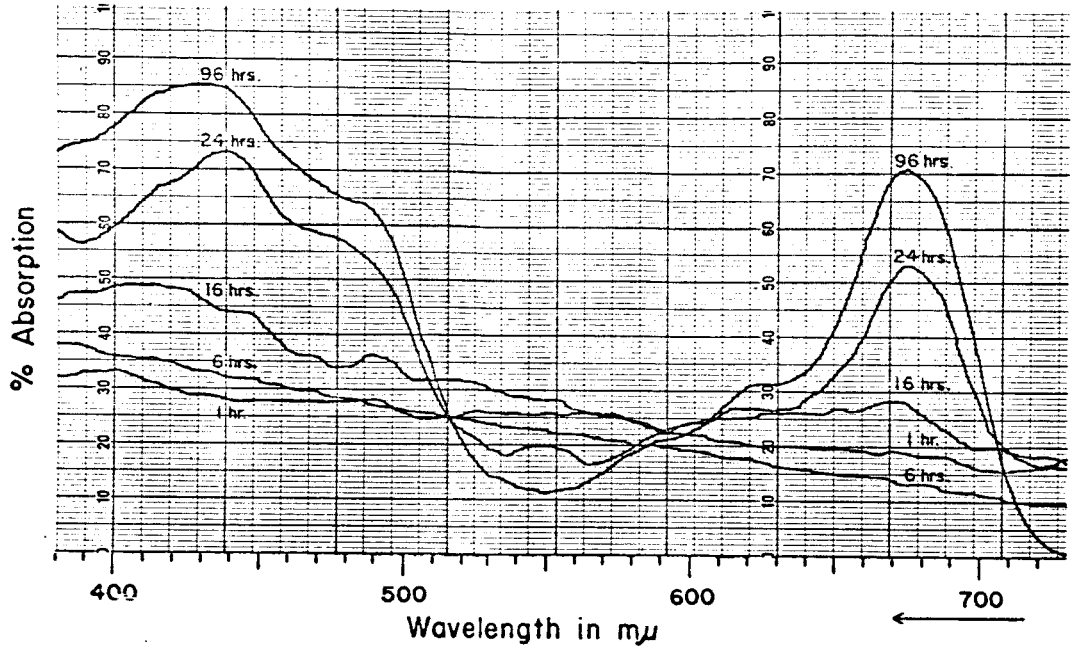

1. Euglena chloroplast spectrum. a, Euglena gracilis chloroplasts.



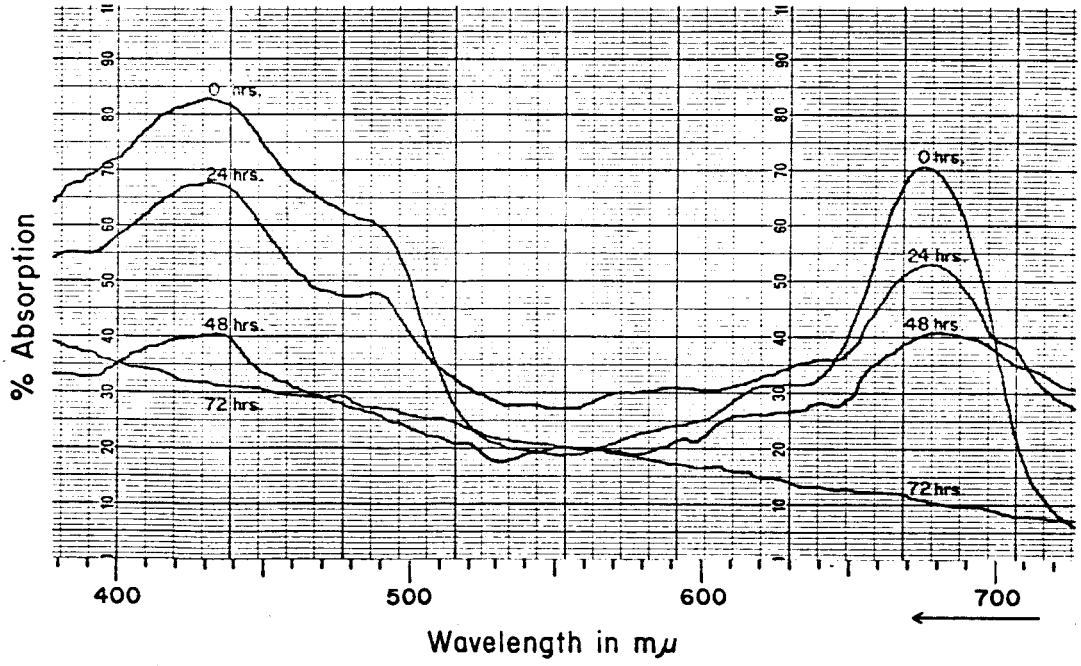

2. U.V. spectrum of chloroplast.

3. Spectrum of dark-grown Euglena.

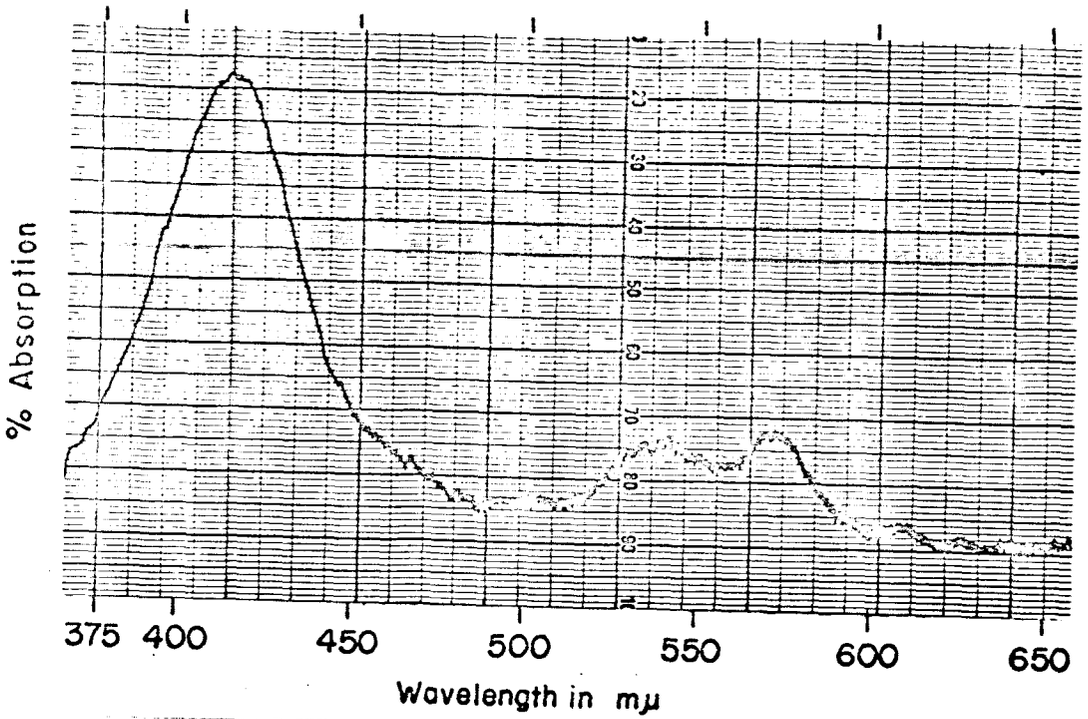




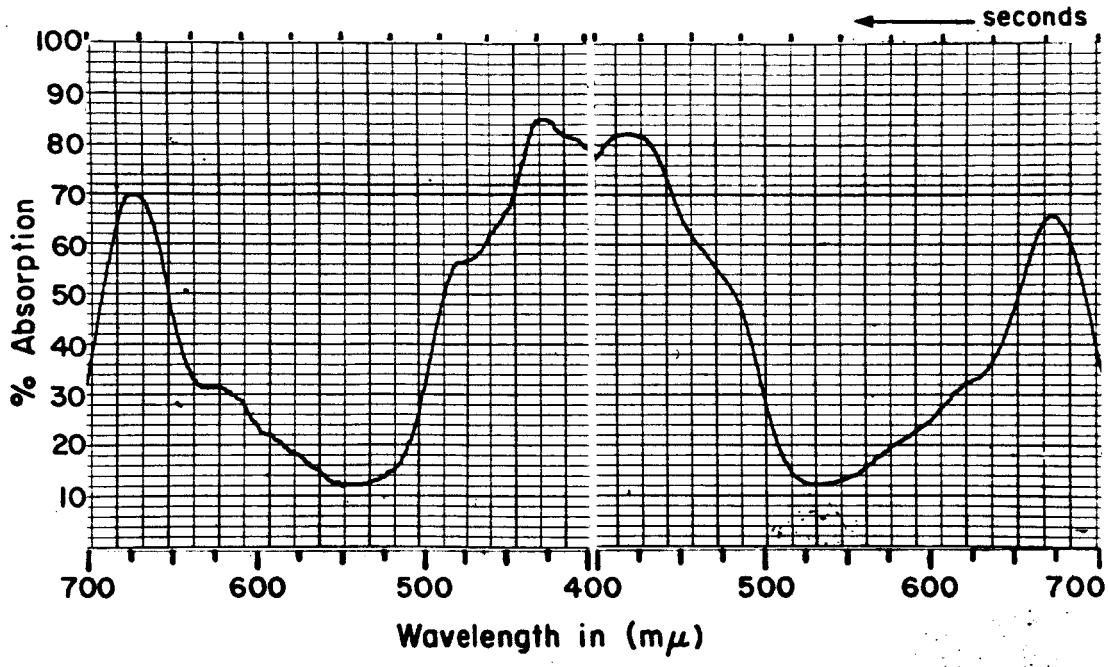

1. Spectra showing the biosynthesis of chlorophyll in the light (Euglena gracilis).




2. Spectra showing the bleaching of chlorophyll with time in darkness.




Spectrum of the frog red blood cell.




Euglena chloroplast spectra.