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Final Report to the Air Force Office of Scientific Research

Contract AF49(638)-762
with the University of Pittsburgh

"Flash Spectroscopy and Flash Fluorimetry in Photosynthetic Studies"

for the period February 1, 1962, to January 31, 1963

A. Scientific Activities

1. Flash spectroscopy of photosynthetic algae

a. Flash-induced absorption changes in the green and blue.

We made a comparative study of the flash induced formation of a pigment, X, absorbing at 515 mμ in different classes of algae. This material X, still unidentified, had previously been observed principally in the green algae. We found that X is formed during saturating flashes also in the blue-green Anacystis nidulans and in the red Porphyridium Cruentum. In Porphyridium, the ratio of the maximum X formation to total chlorophyll was observed to be only 10% of the value of this ratio in the green alga Chlorella. Another measured difference is that the rate constant for disappearance of X in the dark following a flash is five times as great in Porphyridium as in Chlorella. Since the role of X has not yet been established we examined the possibility that it occurs as a non-photosynthetic intermediate, specifically as a consequence of damaging photo-oxidation processes, was examined and we found the latter possibility when we found that X formation in Chlorella could not be prevented in an atmosphere containing less than 0.1% oxygen.

Porphyridium was examined for flash-induced absorption changes at other wave-lengths. The other principal changes were reversible flash-induced bleachings at 460-480 and 475 mμ. These results are in complete analogy with findings of Witt with Chlorella.

b. Absorption changes in the far-red.

A pigment absorbing at 703 mμ has come to occupy a key role in the photosynthetic schemes of Hoch, of Witt, and of Butler. We therefore wanted to examine carefully the conditions under which such a pigment can be reversibly bleached. We expended considerable effort in constructing an optical system with a high differential sensitivity in this part of the far-red. We succeeded in finding conditions for observing small changes in the 703 transmission produced by single flashes with a low intensity of the monitor monochromatic beam.

One of the principal findings at this wave-length region was that most of the apparent change in absorption at 703 mμ is not really a change in absorption but is an experimental artifact. In our original experiments, and in those of Witt and of Hoch, the basic observation was the flash-induced reversible increase in total light reaching a detector placed to receive light directly transmitted by an algal sample. The algae were always illuminated with monochromatic radiation at 703 mμ. We found that

most of the observed transient disappeared if a second monochromator, also set for 703 mμ, was placed between the sample and the detector. These experiments show that the apparent bleaching is due to a temporary increase in the amount of luminescence following the flash. Since the known algal luminescences, either fluorescence or afterglow, have the blue band characteristic of chlorophyll fluorescence, a simple second monochromator such as an interference filter removes most of the artifact.

2. General theory of energy collection in photosynthesis

J. L. Rosenberg has continued his collaboration with Professor James Franck in formulating a model for the energy utilization steps in photosynthesis. A manuscript prepared during the spring and summer of 1962 is now being revised in the light of newer published experimental work. The framework of the conceptual model is as follows:

a. Of the two photochemical steps in photosynthesis, one proceeds by way of the triplet state of an exposed chlorophyll molecule and the second proceeds by way of the excited singlet state of the exposed chlorophyll.

b. Normally, the observed fluorescence in photosynthesizing plants is from the exposed chlorophyll molecule of each unit. The several hundred protected molecules of each unit transfer their energy too rapidly to the exposed site to allow a competing fluorescence from within the unit.

c. A chloroplast may contain varying amounts of a highly ordered phase of chlorophyll. The crystal red shift of the energy levels of this material leads to the result that electronic excitation in the crystalline phase can sensitize the low-lying triplet state of the exposed chlorophyll but not the excited singlet state.

d. In the red and blue-green algae, almost all of the chlorophyll is in the crystalline phase. In the green algae, the percentage of chlorophyll in the crystalline phase depends on culturing conditions, but in any event light absorption above 680 mμ leads almost exclusively to direct excitation of the crystalline pigment.

e. One product of the triplet-sensitized photochemical reaction is the oxidation of a cytochrome permanently attached to the exposed chlorophyll.

f. Energy arriving at the exposed chlorophyll as triplet excitation is less efficient than singlet excitation in promoting the second photochemical step, which is a reduction of the above cytochrome and the oxidation of a subsequent intermediate in the oxygen-liberating chain. The reason for this may be a specifically triplet-sensitized photo-oxidation of chlorophyll by the oxidized cytochrome.

g. The oxidation of a pigment absorbing near 700 mμ is not an essential step in photosynthesis.

h. Afterglow is a dissipative process following photo-ionization within the protected chlorophyll layer.

On the basis of this model we have tried to explain the basic experimental data of the following types:

a. Dependence of fluorescence intensity on incident light intensity.

b. Fluorescent intensity and spectral transients.

c. Wave-length dependence of quantum yield and the Emerson effect.

d. Polarization of fluorescence and dichroism in the chloroplast.

e. The absorption spectrum of the chloroplast and the life-time of the excited states.

f. Dependence of the oxidation state of the essential photosynthetic cytochrome on the wave-length and time of irradiation.

g. Differential spectroscopy of the chloroplast.

h. Transients in oxygen evolution and consumption rates at low and very low intensities.

i. Chromatic transients in the rate of oxygen evolution and fluorescence.

B. Future Plans

With the flash work there are three main problems on which we want to concentrate our attention:

1. Is there a real reversible bleaching of a pigment absorbing near 700 mμ. If so what is the function of this reaction?

2. What is the pigment recognized only by its transient appearance in differential spectroscopy at 515 mμ? Is this pigment related to plastoquinone. Why is it more prevalent in the green algae than in the red and the blue-green?

3. Under what conditions is cytochrome oxidation observed with a time delay with respect to the flash? Is our working hypothesis correct that cytochrome oxidation is instantaneous when it occurs in the mainstream of photosynthesis, but is delayed when it occurs as a means of restoring temporarily bleached chlorophyll during periods of reduced photosynthetic activity.

We will also continue our theoretical work in the systematization of the results of many types of experiments.

C. Administrative Matters

1. Personnel.

Dr. Edgar Insalberg will terminate his full-time connection with the project before September 1, 1963. On that date he plans to begin work with the Space Research Laboratory of the University of Pittsburgh. He will study possibilities of a lunar botany. We hope that he will be available to our project for the training of a successor and for general consultation.

Dr. Jerome L. Rosenberg officially terminates his sabbatical leave on May 31, 1963. He has been spending this period at Technion, Israel Institute of Technology. Because of his absence from the Pittsburgh campus there may be some delay in finding a research associate to succeed Dr. Inselberg.

2. Fiscal affairs.

Expenditures have been very close to those anticipated. The balance remaining on January 31, 1963, was $4,514.06. This will carry over into the 18-month renewal period beginning February 1, 1963.

Respectfully submitted,

(11) April (2) 1963

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Jerome L. Rosenberg
Principal Investigator

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