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Studies at the Department of Terrestrial Magnetism, 5241 Broad Branch Road, Northwest, Washington, D. C. 20015, cover a wide range of subjects in physics, including geomagnetism, cosmic rays, the ionosphere and radio astronomy, geophysics, isotopes, nuclear physics, and problems of biosynthesis.

The Department of Plant Biology, Stanford, California 94305, devotes its attention to the study of photosynthesis, the means by which plants manufacture organic matter, and of the evolutionary mechanisms by which they have reached their great variation in form, size, and distribution.

Research at the Department of Embryology, 115 West University Parkway, Baltimore, Maryland 21210, is directed toward a better understanding of the mechanisms of differentiation, growth, and morphogenesis and the manner in which these processes are coordinated as the fertilized egg is transformed into the functional adult in a variety of species including man.

The Genetics Research Unit, Cold Spring Harbor, New York 11724, studies the biological mechanisms instrumental in the appearance and transmission of specific life traits. The genetic characteristics of bacteriophage and of maize are particular interests. The Unit replaces the former Department of Genetics, active at Cold Spring Harbor from November 1, 1920, to June 30, 1962.
Department of Plant Biology

C. Stacy French
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Stanford, California
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INTRODUCTION

An understanding of how different parts of the photosynthetic mechanism of plants fit together to make up the complete process continues to be the objective of one group in the Department of Plant Biology. As in past years our main concern has been with the functional relationship and the chemical nature of the various pigments that absorb light to supply power for driving the separate reactions.

This aspect of research on photosynthesis is a rapidly developing field that is being taken up by an increasing number of other laboratories. Communication between different groups of workers through meetings, visits, publications, and interlaboratory exchange of active investigators has rapidly expanded. This situation is probably typical of many scientific fields.

Close contact between individuals and groups working on the same subject generally favors the advancement of knowledge, but it does introduce a potentially undesirable factor. A danger to the field of photosynthesis is that through frequent exchange of ideas we may all come to have far too similar concepts about the process and about the relative importance of different areas of investigation. Diversity of opinion in the search for truth is essential at the existing state of knowledge in this subject.

The increase in the pace of scientific advance results in some consequences that are of immediate concern to each individual participating in the endeavor. One of the most obvious is the ever-narrowing specialization that is essential for maintaining professional competence. Over-specialization may reduce the spirit of amateurish enthusiasm for starting work in subjects lying outside the investigator's previous experience, which has great value in a research laboratory. Fresh approaches to fundamental questions without too much detailed knowledge of previous theories, practices, and conclusions have sometimes led to unexpected discoveries and to unconventional interpretations. As a field gets more complex the need for the fresh mind increases, yet the chance for real success by the amateur continually diminishes because of the sheer volume of relevant known facts and because the tools of the trade become harder to master.

Strangely enough the number of significant questions awaiting investigation, rather than decreasing, grows ever larger, for each successful study raises more problems at a deeper level of understanding than it solves at the previously attainable level. In the field of photosynthesis there is an abundance of new concepts to be tested, old theories to be revised, new effects to be explained, and always the certainty of new discoveries arising as the result of improved experimental methods with different and unusual plant species.

A large part of research on the mechanism of photosynthesis is now concentrated on attempts to understand the Emerson enhancement effect: the increase in photosynthetic rate achieved when two appropriate colors of light are given together as compared with the sum of the rates from the two colors given separately. The discovery of this effect was one of the main reasons for believing that two pigment systems are involved in photosynthesis. Measurements of enhancement have become the most widely used means for studying the nature and the interactions of the two systems.

Most of the experiments on enhancement have been made by measuring oxygen evolution. While visiting the Department in the summer of 1962, Professor Martin Gibbs with his collaborators, Dr. Charles A. Fewson and Mr. Marvin D. Schulman, all from Cornell University, investigated the uptake of
carbon dioxide, finding enhancement effects similar to those known from oxygen measurements. They also measured the reduction of the hydrogen-transfering material, TPN, and the formation of high-energy phosphate, ATP, from inorganic phosphate by isolated chloroplasts. Both these photochemical reactions are considered to be intermediate steps of photosynthesis. The enhancement effect was not observed for either of them. The lack of enhancement in some of the individual steps indicates that the effect may be due to changes in the steady-state concentration of intermediate products rather than to an influence on the efficiency of the photochemical reactions themselves.

During the report year measurements of the rate of photosynthesis have been made with short light flashes of colors chosen to activate specific pigment systems of algae. In the red alga *Porphyridium* we found the response to single short flashes when given with or without continuous background light to show the enhancement effect in two distinctly different ways. Green flashes with red background light gave an immediately increased initial rate of oxygen evolution over that obtained without the continuous red light. After the flash the rate declined just as rapidly with or without the red light. When red flashes were given with a continuous green background light, however, a different kind of enhancement was found. The initial rate of oxygen evolution was no greater than without the green background light, but the evolution of oxygen continued longer after the flash. Thus, either an increased initial rate or a prolongation of the time of gas evolution gives an enhancement effect, but the kinetics in the two responses are very different.

Variation of the time schedule of repetitive flashes of red or green light affects the photosynthetic rate differently. With green light, lengthening the flash time and reducing the dark time has less effect on the photosynthetic rate than a similar change in the time distribution of red light flashes. Such experiments are being continued to study the interaction of the two pigment systems of photosynthesis.

A theory proposed some years ago by Hill and Bendall ties together in a logical way some of the known intermediate reactions of photosynthesis on the basis of their oxidation-reduction potentials. Many different investigators have elaborated on this model. Witt has used its basic framework to correlate the chemical events indicated by the small changes of light absorption measurable in various parts of the spectrum when photosynthetic cells are illuminated. Essentially the same scheme has also been used by Arnon, Duysens, and others to show how the interrelations of numerous partial reactions, observed in isolated chloroplasts and in whole cells, can be correlated into a reasonable picture of the part of photosynthesis that is closely related to the photochemical function of the two pigment systems.

Although the details of the current concepts and the language used to describe them vary in different laboratories, it is generally agreed that the oxygen-evolving reaction is more closely connected with the activation of the accessory pigment system than with the long-wavelength chlorophyll system. Furthermore, activation of the accessory pigment system is widely believed to be the initial act starting off the series of reactions. Some of our recent results at first sight appear to be in sharp contrast to this point of view.

We may look at photosynthesis as a system driven by two inputs: (1) light absorbed by or transferred to long-wavelength chlorophyll $a$, and (2) light absorbed by the accessory pigment system. We may then ask in which order these two distinct energy inputs act on the resulting chain of chemical events. Is a product of one photochemical reaction used as a substrate for the other one; and, if so, does the accessory pigment reaction or
On the other hand the two light inputs may equally well be considered to act simultaneously and independently on the two systems. The model that we used previously for describing the various shapes of induction time course curves obtained in *Porphyridium* assumed that the two pigment systems acted in a parallel fashion rather than one after the other.

The obvious conclusion at present from the recent flashing light experiments in red algae is that material made by chlorophyll *a* is used up by the accessory pigment system as oxygen is produced. This conclusion, if taken at its face value, would reverse the accepted time sequence of the operation of the two photochemical reactions by placing the long-wavelength chlorophyll *a* system at the starting point.

There is, however, a consideration that prevents this obvious interpretation of the experimental results from being a necessary conclusion, namely, the fact that a cycling system having a certain dynamic equilibrium concentration of components during steady-state photosynthesis in the light may be changed during a dark period to a different set of concentration levels of the components. Thus the result of chlorophyll *a* activation may only be the necessary readjustment of the various pool sizes of intermediates from their dark equilibrium values to the different values required for the efficient operation of the system in the equilibrium condition.

One of our main objectives is to ascertain the extent to which the parallel reaction model or a series-type model can account for the effects observed in the flashing light experiments and can relate them to other known properties of the photosynthetic system.

The properties of the different forms of chlorophyll *a* such as their comparative extractability by organic solvents and their sensitivity to heat, light, and detergents have been further studied by Dr. Brown in the past year. The longer-wavelength forms of chlorophyll are more susceptible to these destructive treatments than *C*₆₇₂.

A clear separation of the function of several of these forms of chlorophyll was achieved by Dr. Fork in collaboration with Professor Witt and Dr. Müller at Marburg. They found that the small increase in light absorption of spinach chloroplasts at 515 mû is caused by the accessory pigment system composed of chlorophyll *b* and the form of chlorophyll *a* absorbing at 674 mû. On the other hand the absorption decrease of chlorophyll *a* measured at 433 mû was found to be caused by the two long-wavelength forms of chlorophyll *a* absorbing at 682 and 695 mû.

The kinetics of several of the partial reactions of photosynthesis have been studied by rapid electrical recording methods. Thus Nishimura and Chance have measured phosphate uptake rapidly by following pH changes. Since other reactions than phosphate uptake can also give pH changes, the conditions under which this method is used must be carefully chosen. An attempt is being made by Dr. Smith to develop an electrode as specific as possible for phosphate ion itself. A silver electrode coated with a thin layer of a dense suspension of silver phosphate has been found to give excellently reproducible measurements of very small amounts of phosphate in buffered solutions. Unfortunately the substances present in phosphorylating suspensions of chloroplasts preclude the use of this system for its intended application. Experiments with other electrode systems are in progress with the hope of developing a general-purpose electrode for phosphate determination in biological systems.

Dr. Jerome A. Schiff of Brandeis University visited the Department to work on one aspect of his program on the development of the photosynthetic apparatus in *Euglena*. He found that when the dark-grown alga is transferred to
light the ability to produce oxygen develops in about 4 hours; up to this time light accelerates oxygen consumption by the alga. The Department profited greatly from Dr. Schiff's interest in experimental techniques. While here he improved the performance of the equipment for measuring action spectra and added an automatic device to keep the wavelength sweep speed at the optimum value.

Purple bacteria utilize light for a form of photosynthesis that produces material for their growth but without evolving oxygen like green plants. These bacteria are particularly useful in the study of photosynthesis because both their photosynthetic products and their pigment systems contrast with those of green plants. In the dark, photosynthetic bacteria, like other plants, consume oxygen when it is available. If they are exposed to light the oxygen uptake is strongly inhibited, presumably by the diversion of respiratory substrates into the photosynthetic pathway. Dr. Fork, working at Utrecht in collaboration with a former Institution Fellow, Dr. Goedheer, and Professor Thomas, investigated this effect of light on purple bacteria. They found that the action spectra of the light inhibition of respiration is a near match to those previously known for bacterial photosynthesis, for the excitation of fluorescence in bacteriochlorophyll, and for the effect of light in orienting the swimming of the bacteria. In all these effects bacteriochlorophyll is active while the carotenoids are sometimes only partly functional.

The formation of chlorophyll from protochlorophyll in plants grown in the dark requires light. Whether this reaction is a single-step photochemical reaction or whether light initiates a series of reactions leading to the final product—chlorophyll—has long been discussed. This year Mr. Axel Madsen, visiting the Department from the Royal Veterinary and Agricultural College, Copenhagen, found that the transformation of protochlorophyll to chlorophyll was complete in leaves in the shortest time that could be measured, 4 milliseconds. With protochlorophyll holochrome solutions as prepared by Dr. Smith, which do not scatter light, a different optical system was used, in which the time resolution could be reduced to 1 msec. After this time no more transformation was seen. It is therefore certain that if any dark reactions are involved they are completed within a thousandth of a second.

A green alga, apparently a *Stichococcus*, was found growing in an unusual habitat—a 1 M solution of MgSO₄ on the laboratory bench. When Dr. Brown isolated this strain in pure culture she found it to contain an unusually high proportion of chlorophyll *b*, which makes it particularly useful for comparative studies of pigment functions. Enhancement effects and the action spectrum for this alga are under investigation by Dr. Govindjee.

During the past year some time was spent on a project far removed from our normal activities. It came about because we thought we saw a way to improve one of the standard methods for making largescale topographic maps of small areas. The idea was to use an optical range finder for plane table mapping in such a way that a point observed through the range finder telescope is automatically located on the map by an index marker. All calculating and plotting are done by the instrument itself. The device should make it possible for one or two men to do much more rapidly the work now requiring a survey party of three or more.

An experimental instrument incorporating these principles has been built. Although tests and modifications are still in progress, it appears that distance accuracy within 1 foot and elevation accuracy of about 0.1 foot will be attainable at a scale of 1 inch = 10 feet. Other scales are also possible.

Parts of the accumulated data from long-term genetic and transplant experi-
ments on *Achillea* and *Mimulus* have been analyzed with the help of an electronic computer to determine whether the principle of genetic coherence, an important concept that emerged from earlier work on *Potentilla*, applies to other groups of higher plants as well. The results, in conclusive agreement with those found in *Potentilla*, indicate that the principle is a general one.

Genetic coherence is the tendency for sets of characters that distinguish one recognizable biological entity from another to be inherited together as a block rather than to segregate separately in a purely random Mendelian fashion. Coherence results from the fact that the vast majority of characters distinguishing biological entities, rather than being determined by single genes located on a single chromosome, are controlled by multiple genes, the components of which may be distributed over more than one chromosome. The principle of coherence is important, because it not only provides a rational genetic basis for the differentiation of ecological races, subspecies, and species but also orients studies aimed at discovering physiological mechanisms underlying natural selection.

The existence of genetic coherence has been difficult to prove experimentally because of the wide segregation of character combinations typically observed in the second-generation progeny of crosses between contrasting climatic races. The overwhelming array of new combinations of characters tends to obscure the important effects of coherence. Measurements of specific characters of individual plants in fairly large samples of segregating hybrid populations when tested as cloned transplants at the altitudinal transplant stations provide the data essential for determining the existence of genetic coherence and its role in natural selection.

By means of controlled growth cabinets it is now possible to grow alpine forms of *M. lewisii* at Stanford so that measurements on rates of photosynthesis can be made. Preliminary work indicates that photosynthetic rates of high-altitude races of *M. lewisii* are distinct from those of races of *M. cardinalis* that range at lower altitudes. The data are yet too incomplete to allow conclusions.

Methods for establishing and growing tissue cultures of different races of *Mimulus* are being perfected so that the comparative growth and development of parts of cloned individuals can be studied under sterile, controlled conditions. This work is being closely coordinated with the studies at the transplant stations, in the controlled cabinets, and by photosynthetic measurements.

Biochemical studies on key clones of *Mimulus cardinalis* used in the photosynthetic measurements have been started by Mr. D. McMahon, a graduate student working with Professors Olmsted and Bogorad at the University of Chicago. Mr. McMahon is making a comparative study of enzyme systems concerned with assimilation and respiration in different climatic races. His work, which is being carried on independently, can be coordinated with our investigations through the use of the same cloned plants having identical genetic composition.

Dr. Clausen has devoted considerable effort to the tree lines in the Harvey Monroe Hall Natural Area, in which the Timberline station is situated. A field study of altitudinal segments representing different slope exposures discloses characteristic differences both in the composition of genetic variants and in the over-all levels of tolerance to high altitudes of three distinct species of conifers, *Tsuga mertensiana*, *Pinus murrayana*, and *P. albicaulis*.

Miss Yvonne Aitken, senior lecturer in the School of Agriculture at the University of Melbourne, Australia, is spending part of her sabbatical year at the Department, studying growth and flowering responses of 30 varieties of important crop plants at the Stanford, Mather, and Timberline transplant stations. This is part of a larger investigation on the com-
comparative development of early and late crop varieties at different latitudes and altitudes. Other plantings are located at the University of Melbourne, the University of Hawaii, Oregon State College, the University of Saskatchewan, and the Welsh Plant Breeding Station at Aberystwyth.

Dr. Theodosius Dobzhansky is continuing evolutionary studies on native Drosophila populations across the Sierran transect, using the Mather transplant station as headquarters for his operations during part of the summer of 1963. This is part of a long-term survey in progress for many years.

PERSONNEL

Biochemical Investigations

Staff: C. Stacy French, Director, Jeanette S. Brown, David C. Fork, James H. C. Smith, Emeritus

Visiting Investigators: Charles A. Fewson, Martin Gibbs, Govindjee, Rajni Govindjee, Axel Madsen, Jerome A. Schiff, Marvin D. Schulman

Technical Assistants: Robert A. Clair, Harriet M. Fulk, David N. Lion, D. David Perfect, Devender C. Reddy

Experimental Taxonomy

Staff: Jens C. Clausen, Emeritus, William M. Hiesey, Harold W. Milner, Malcolm A. Nobs

Visiting Investigator: Yvonne Aitken

Summer Research Assistant: Andrew N. Lenz

Technical Assistants: Frank Nicholson, Kathie A. Picken

Clerical Assistant: Marylee H. Eldredge

Gardeners: Joseph S. Chang, Emmett R. Clagg, Erica Duveneck, Wesley B. Justice

Administrative Assistant

Wiley Knight, Jr.

Mechanic

Richard W. Hart

Custodian

Jan Kowalik

Wesley B. Justice retired on April 30, 1963, after 16 years as gardener with the Department.

Dr. Jeanette S. Brown and Dr. David C. Fork attended the Photosynthesis Colloquium at Gif-sur-Yvette in July 1962. In August 1962 Dr. Brown attended a colloquium on the metabolism of chlorophyllous pigments in the leaf, at Centre de Recherches de Gorsem, St. Trond, Belgium. At the end of June 1963 she left for a 4-month period of work with Dr. J. Duranton of the Département de Biologie, Commissariat à l’Énergie Atomique, Saclay, on the chemical separation of chlorophyll holochrome. The remainder of the coming year she plans to spend in the Philippines collecting algae for future use in studies of pigment function.

This spring Dr. David C. Fork returned from a year in Europe, where he worked in three laboratories. After a month’s visit at Laboratoire de Photosynthèse in Gif-sur-Yvette near Paris he spent 6 months in the Physical-Chemical Institute of Professor H. Kuhn at the Philips University of Marburg, where he worked with Professor H. T. Witt and Dr. A. Müller on the action spectra for the production of fast absorption changes associated with different light reactions of photosynthesis. He spent the remainder of his time in Professor J. B. Thomas’s Biophysical Research Group of the State University at Utrecht. There he collaborated with Dr. Goedheer on a study of the light-induced inhibition of respiration in photosynthetic purple bacteria and also observed Dr. Goedheer’s apparatus for measuring the luminescence of photosynthetic organisms.

Dr. Jens Clausen lectured at the National Science Foundation Summer Institute for College Teachers at Vanderbilt University in July 1962 on the function of populations in the evolution
of species and their adjustment to environment. In April 1963 he attended the annual meeting of the National Academy of Sciences in Washington, D. C. There, and at the University of Tennessee, Knoxville, and at Vanderbilt University, Nashville, he presented papers and seminars on the distribution and evolution of trees.

After his work as a visiting investigator at the Royal Agricultural College at Uppsala, Sweden, last year, Dr. Malcolm Nobs took part in an international botanical symposium held at Prague during July 1962.

Dr. William M. Hiesey participated in an international symposium on the environmental control of plant growth at Canberra, Australia, during August 1962, held in connection with the official opening of the Controlled Environment Research Laboratory (Ceres) built by the Division of Plant Industry of the Commonwealth Scientific and Industrial Research Organization. The symposium on environmental control was preceded and followed by meetings at the University of Sydney and the University of Melbourne dealing with various aspects of plant science, including physiology, genetics, and microclimatology, sponsored independently by the Australian and New Zealand Association for the Advancement of Science and by the CSIRO.

BIOCHEMICAL INVESTIGATIONS

EXPERIMENTS WITH COLORED LIGHT FLASHES

C. S. French

Some of the more useful information for the interpretation of the kinetics of photosynthesis over the past few decades has come from the study of rates of photosynthesis in flashing light. From such measurements, the concept of the "photosynthetic unit" has developed and the time constants for the rates of some of the enzymatic steps in photosynthesis have been determined by various workers.

Many of these investigations have been carried out with great experimental ingenuity, high precision of measurement, and extensive theoretical insight. Except for the recent work of Whittingham, these flashing light experiments were done before it was known that two separate photochemical reactions participated in the process, and so no attention was paid to the influence of wavelength on the rates of photosynthesis in flashing light. We are therefore studying the kinetics of photosynthesis in flashing light of different colors chosen to activate certain pigments preferentially.

Last year we reported that, in the red alga Porphyridium, green light flashes of a few seconds' duration gave more oxygen if preceded by a flash of red light lasting several seconds. The production of oxygen from long red flashes, however, was not enhanced by a previous green flash. In those experiments the time course of oxygen evolution from each flash, rather than steady-state rates, was measured. The material produced by activating chlorophyll a with red light disappeared in the dark with a half-life of about 18 seconds.

We reasoned by analogy that perhaps a substance might be produced by green light that could enhance oxygen evolution by a succeeding red flash but that it might have too short a survival time to be detected in those experiments.

This year we tested that hypothesis by measuring the steady-state rate of oxygen evolution from paired flashes of the two colors given repeatedly. The flashes themselves were much shorter, and the dark interval between them was greatly reduced. Experiments were made with two timing schedules: flash time 10 or 4 msec; time between flashes 50 or 10 msec; time between pairs of flashes 500 or 430 msec.
Fig. 1. The variation of photosynthetic rate of *Porphyridium* with sector speed in two colors of flashing light. Conditions: artificial sea water, 5 per cent CO₂ in air, about 23°C, sector opening about 1.2 per cent of circumference.

Dark intervals between the closely spaced pairs of flashes were long enough to allow for the decay of the hypothetical product of the green light but short in comparison with the lifetime previously found for the product of the red light. The idea was that, if an enhancing green light product with a very short lifetime existed, more oxygen would be produced per minute if the green flash came just before rather than after the red flash. No such effect was found. We therefore conclude that, in *Porphyridium*, material made by chlorophyll *a* enhances the production of oxygen by green light but that the opposite situation does not exist. No evidence was found for the presence of a substance made by green light that can enhance photosynthesis subsequently in red light.

Among other effects that have been observed with various regimes of colored light flashes in *Porphyridium* is the difference in the way the steady-state rates of photosynthesis in red and in green light depend on the flash duration or on the dark time between flashes. In these experiments a sector with a single opening about 1.2 per cent of the circumference was used. It rotated about 10 times per second, and the intensities of red and of green light were adjusted to give the same rates of photosynthesis at this high speed. Measurements were then made with slower speeds, but with the cells always having the same amount of light per minute and the same intensity as at the faster speed. At lower speeds both the light and the dark periods were of longer duration.

The results are shown in figure 1. With green light the rate declined with decreased sector speed much less rapidly than with red light. The implications of this effect are not yet evident, beyond showing a difference in the kinetics of photosynthesis in red and in green light on a short time scale.

In other experiments the photosynthetic rate with high and low sector speed both with red and with green light was measured at several intensities. In figure 2 the ratio of the rates at low and at high speeds is plotted for the two
colors. The high speed was 9.5 rps, which gave a light exposure time of 2.3 msec; the low speed was 0.49 rps, giving 49-msec exposures. The amount of light per minute was the same for both speeds. After measurements at two sector speeds for each color the intensity was changed to a new value. The rate of photosynthesis with the high-speed flashes was used as a measure of effective light intensity. It is the abscissa of figure 2.

At the lowest intensity of green light flashes the slow and fast speeds were equally effective; as the intensity increased, photosynthesis at low speed became less effective than at the higher speed. With red flashes the rates at low speed were far below those at high speed. It looks as though higher intensities of green would have given results approaching the same low-speed ratio as was found for red light if sufficient intensity had been available.

In a different type of experiment unexpected effects were found when the oxygen production from single short flashes was studied. Red or green flashes lasting about 50 msec were given at 46.8-second intervals both with and without continuous background illumination, as shown in figure 3. Because of the long time between flashes the time course for
rate of oxygen evolution from each flash appeared on the record. The rate reached its peak value in about 2 seconds and dropped halfway back to the base line in about 6 seconds.

The lag in the apparatus response is greater for the drop than for the rise, because the drop depends on oxygen removal by diffusion through the cellophane membrane over the cells. The instrumental lag for the drop would have a half-time of about 3 seconds for a sudden stopping of oxygen evolution (Year Book 61, p. 346). After the flashes with no background light the observed lag was about 6 seconds for either green or red, showing an appreciable continuation of oxygen production after the flash.

When either red or green flashes were given on a background of the opposite color more oxygen evolution was found from each flash than without the background light. The shape of the time course curve for oxygen evolution following each flash with complementary background light, however, was very different.

The green flashes had about twice the peak rate when given on a continuous red background illumination, as seen in the upper right of figure 3. Here the drop back to the base line was as rapid as without the continuous red light.

By contrast the red flashes when given on the continuous green background showed no increase in the peak rate but the rate dropped back to the base line more slowly, thus also giving more total oxygen than without the background light. The half-time was about 10 seconds. The total oxygen evolution from each flash, that is, the area under the rate curve, was larger for either color of flash with complementary background light, thus showing the enhancement effect either way but with very different kinetics.

In summary, the enhancement of oxygen evolution from a single flash produced by the background light can appear in two different ways: through an increase of the initial rate, or by prolonging the time during which oxygen evolution continues after the flash.

In the upper part of figure 3 the peak rates of oxygen production from green flashes given during the few minutes following the red light exposure are smaller than before the continuous red light exposure. This is just opposite to the effect expected from last year's work with longer flashes of green light following flashes of red light.

Thus we see that many factors controlling the rate of photosynthesis with different regimes of light exposure to various wavelengths are still unknown. These phenomena, which appear to offer promising ways to study the two pigment reactions in photosynthesis, are being investigated further by Drs. Govindjee and Rajni Govindjee.

Studies on the Enhancement of Some Photochemical Reactions Carried Out by Whole Cells and by Cell-Free Preparations

Martin Gibbs, Charles A. Fawson, and Marvin D. Schulman

One of the first indications that two different photochemical reactions are involved in photosynthesis by algae and higher plants came from the enhancement studies of Emerson. It had been known for some years that photosynthesis is less efficient in the far-red (beyond about 695 mμ) than at shorter wavelengths. Emerson discovered that there is a synergistic effect on photosynthetic oxygen evolution if red light and light of a shorter wavelength are presented simultaneously. The enhancement of photosynthesis may be considered in two ways: either as a mutual enhancement or as an increase in the efficiency of only the long-wavelength light. The effect can therefore be described by two ratios:

**Mutual enhancement (E total) =**

\[
\frac{\text{Rate} (\lambda \text{ short} + \lambda \text{ long})}{\text{Rate} \lambda \text{ short} + \text{Rate} \lambda \text{ long}}
\]
Red enhancement (E red) = 
\[ \frac{\text{Rate} (\lambda_{\text{short}} + \lambda_{\text{long}}) - \text{Rate} \lambda_{\text{short}}}{\text{Rate} \lambda_{\text{long}}} \]
where the parentheses indicate simultaneous exposure to short and long wavelengths.

French, Brown, and Fork (Year Books 60, pp. 351–362, and 61, pp. 345–357) have studied the photostimulated oxygen uptake carried out by algae. It is clear that this phenomenon could interfere with determinations of photosynthetic enhancement as measured by oxygen evolution. Apparently this light-dependent oxygen uptake is not accompanied by carbon dioxide evolution and is thus quite distinct from respiration; rather, it is a side effect of the photochemical mechanism (Year Book 60, p. 354). We therefore wished to study the enhancement of carbon dioxide assimilation by whole algal cells, since most previous enhancement studies have been made on oxygen evolution. By measuring the assimilation of C\(^{14}\) carbon dioxide we thought that we might minimize the possibility of obtaining spurious results caused by side reactions. We also wished to see whether the various chloroplast reactions such as oxygen evolution and TPNH and ATP formation show an enhancement effect.

**Algae.** The blue-green alga Anacystis nidulans, the red alga Porphyridium cruentum, and the green alga Scenedesmus obliquus were harvested from actively growing cultures and resuspended in artificial sea water before use. No damage from sea water to the Scenedesmus appeared within the hour needed for the experiments.

**Chloroplasts.** Swiss chard and spinach were grown in the garden at Stanford, and some additional plants were purchased from local stores. Mature leaves were rinsed with distilled water and chilled in an ice-slush for 20 minutes. All further steps were carried out in a cold room in dim light. The midribs were removed, and the leaf blades were chopped into small fragments. The fragments were then ground with sand in a solution containing 0.35 M NaCl and 0.02 M tris buffer, \(pH\) 7.6. The slurry was strained through 8 layers of cheesecloth and centrifuged at 200g for 2 minutes. The supernatant fluid was then centrifuged at 1000g for 10 minutes. The precipitated chloroplasts were resuspended in 0.35 M NaCl, 0.02 M tris, \(pH\) 7.6, and stored at about 4°C in the dark.

**Particle preparations from Anacystis** were made by freeze-drying as described elsewhere (Nature, 198, 88, 1963).

**Measurement of oxygen evolution.** The rates of oxygen output by chloroplasts or algae were measured with a platinum electrode covered with a thin layer of Teflon as described by Dr. Fork (Year Book 61, p. 343). The standard circulating fluid contained 0.4 M sucrose, 0.01 M NaCl, and 0.05 M KH\(_2\)PO\(_4\)-K\(_2\)HPO\(_4\) (\(pH\) 7.4). The illumination was provided either from a monochromator with supplementary stray light filters or from tungsten lights with interference and stray light filters as described in the Year Books of the last four years.

**Measurement of carbon dioxide assimilation.** The reaction mixture for measuring carbon dioxide fixation contained algal suspension (10\(^6\) to 10\(^6\) cells in artificial sea water, \(pH\) 6.9, without HCO\(_3^-\)); NaHCO\(_3\), 10 micromoles (containing approximately 10 microcuries C\(^{14}\)); total volume 1.5 ml. After various times of illumination 0.10-ml aliquots were removed and added to 0.90 ml of 0.25 N HCl in 95 per cent ethanol contained in planchets. The samples were evaporated to dryness, and the radioactivity was determined. The reaction mixture was contained in a Beckman 1-cm-light-path cuvette.

Illumination was provided from two opposite sides by projector lamps. The filaments of the lamps were imaged on the cuvette faces. The far-red light was obtained by passing the beam through a 12-cm water filter, a Bausch and Lomb interference filter of wavelength as indi-
icated in tables 1–4, and Corning glass filter 2030. The short-wavelength beam was obtained by passage through a 5-cm 1 per cent w/v CuSO₄ solution, an appropriate Bausch and Lomb interference filter, a Corning glass filter 2408, 2418, 3387, or 3484, and an infrared blocking filter. Beam intensities were controlled by variable voltage transformers. Shutters were interposed between the filters and the cuvette.

TPNH formation. The reduction of TPN by chloroplasts or by *Anacystis* preparations was measured in reaction mixtures containing tris-HCl buffer, pH 7.6, 24 micromoles; MgCl₂, 4 μmoles; KH₂PO₄-K₂HPO₄, pH 7.5, 1.54 μmoles; ADP, 2 μmoles; TPN, 1.5 μmoles; photosynthetic pyridine nucleotide reductase purified from spinach, 1 unit; chloroplasts or *Anacystis* particles containing about 30 μg chlorophyll; total volume 2 ml. After illumination under the same conditions as used for measuring carbon dioxide assimilation, the reaction mixtures were centrifuged in the dark at

**TABLE 1. Oxygen Evolution by Intact Algae**

<table>
<thead>
<tr>
<th></th>
<th>Expt.</th>
<th>627 mμ</th>
<th>694 mμ</th>
<th>627 mμ + 694 mμ</th>
<th>E Red</th>
<th>E Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anacystis nidulans</em></td>
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<td>33</td>
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</tr>
<tr>
<td></td>
<td>2</td>
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<td>12</td>
<td>35</td>
<td>1.58</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
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<td>9</td>
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<td></td>
<td>3</td>
<td>14</td>
<td>3</td>
<td>22</td>
<td>2.66</td>
<td>1.29</td>
</tr>
</tbody>
</table>

**TABLE 2. Carbon Dioxide Fixation by Intact Algae**

<table>
<thead>
<tr>
<th></th>
<th>Expt.</th>
<th>567 mμ</th>
<th>694 mμ</th>
<th>567 mμ + 694 mμ</th>
<th>E Red</th>
<th>E Total</th>
</tr>
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<tbody>
<tr>
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<td>3283</td>
<td>3.66</td>
<td>1.52</td>
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<tr>
<td></td>
<td>2</td>
<td>3252</td>
<td>728</td>
<td>4885</td>
<td>2.22</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1954</td>
<td>406</td>
<td>4485</td>
<td>6.24</td>
<td>1.90</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>1</td>
<td>132</td>
<td>36</td>
<td>248</td>
<td>3.0</td>
<td>1.48</td>
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<tr>
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<td>324</td>
<td>162</td>
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<td>1.89</td>
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<td><em>Scenedesmus obliquus</em></td>
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<td></td>
</tr>
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<td>166</td>
<td>11,768</td>
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</tbody>
</table>
4°C. TPNH formation was determined by absorbancy measurements at 340 mμ.

Enhancement of oxygen evolution by algae. Table 1 shows, in confirmation of the results obtained by many workers, that there was a consistent enhancement of the rate of oxygen evolution by whole cells of Anacystis and Scenedesmus when red and short-wavelength lights were presented together.

Enhancement of carbon dioxide assimilation by algae. An enhancement of the rate of carbon dioxide uptake could be observed with the green, red, and blue-green algae studied, as shown in table 2. The similarities of the degrees of enhancement shown by carbon dioxide fixation and by oxygen output suggest that previous estimates of enhancement have probably not been greatly affected by the photostimulated oxygen uptake, provided, of course, that there is definitely no carbon dioxide output associated with the light-activated oxygen uptake. Figure 4 demonstrates that the degree of enhancement is markedly affected by the ratio of the two lights. The precise significance of this is not clear, although it is undoubtedly a reflection of the pigment complement of the organism. Myers and Graham (Plant Physiol., 38, 105, 1963) have presented a similar type of curve for the effect of ratio of incident energies on the enhancement of oxygen evolution by Chlorella. Figure 4 also illustrates the variation in the value obtained for enhancement according to the method of calculation.

Lack of enhancement of the Hill reaction. Table 3 illustrates experiments in which we attempted to obtain enhancement of the rate of oxygen production by Swiss chard and spinach chloroplasts and by Anacystis particles. Under no conditions were we able to find a positive enhancement. Experiments were carried out with ferricyanide as the electron acceptor, and we also studied the endogenous chloroplast reaction described in detail by Dr. Fork (Year Book 61, p. 334). A wide range of wavelength combinations, relative and absolute light intensities, and different particle preparations all gave uniformly negative results.

A few experiments were also carried out on TPN reduction by spinach chloroplasts and by Anacystis particles. No enhancement could be observed, a finding that confirms preliminary results obtained in collaboration with Dr. C. C. Black and Dr. S. A. Gordon at the Argonne National Laboratory. In these earlier experiments we did notice one consistent and very pronounced interaction of monochromatic lights, namely an inhibitory effect of prior exposure to light of wavelength longer than 700 mμ on TPNH and ATP formation and carbon dioxide fixation by spinach chloroplasts at shorter wavelengths.

Valid enhancement studies on ATP formation by chloroplasts do not appear to be possible at the moment because of the sigmoid response of ATP formation to increasing light intensity (Turner, Black, and Gibbs, J. Biol. Chem., 237, 577, 1962).
### Table 3. Effect of Combinations of Monochromatic Light on the Hill Reaction

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Acceptor</th>
<th>650 m(\mu)</th>
<th>694 m(\mu)</th>
<th>700 m(\mu)</th>
<th>650 m(\mu) + 694 m(\mu) + 700 m(\mu)</th>
<th>E Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ferricyanide</td>
<td>3.5</td>
<td>2.5</td>
<td>---</td>
<td>6</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>Ferricyanide</td>
<td>15</td>
<td>---</td>
<td>8</td>
<td>22</td>
<td>0.96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Acceptor</th>
<th>650 m(\mu)</th>
<th>694 m(\mu)</th>
<th>700 m(\mu)</th>
<th>650 m(\mu) + 694 m(\mu) + 700 m(\mu)</th>
<th>E Total</th>
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<tr>
<td>1</td>
<td>---</td>
<td>7</td>
<td>15</td>
<td>---</td>
<td>23</td>
<td>1.05</td>
</tr>
<tr>
<td>2</td>
<td>---</td>
<td>23</td>
<td>10</td>
<td>---</td>
<td>29</td>
<td>0.88</td>
</tr>
<tr>
<td>3</td>
<td>Ferricyanide</td>
<td>16</td>
<td>8</td>
<td>---</td>
<td>24</td>
<td>1.00</td>
</tr>
<tr>
<td>4</td>
<td>Ferricyanide</td>
<td>18</td>
<td>---</td>
<td>13</td>
<td>30</td>
<td>0.97</td>
</tr>
<tr>
<td>5</td>
<td>Ferricyanide</td>
<td>10</td>
<td>---</td>
<td>4</td>
<td>14</td>
<td>1.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Acceptor</th>
<th>627 m(\mu)</th>
<th>694 m(\mu)</th>
<th>627 m(\mu) + 694 m(\mu)</th>
<th>E Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ferricyanide</td>
<td>2</td>
<td>7</td>
<td>9</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>Ferricyanide</td>
<td>7</td>
<td>1</td>
<td>7</td>
<td>0.88</td>
</tr>
<tr>
<td>3</td>
<td>Ferricyanide</td>
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<td>2.5</td>
<td>10</td>
<td>0.87</td>
</tr>
<tr>
<td>4</td>
<td>Ferricyanide</td>
<td>7</td>
<td>6</td>
<td>12.5</td>
<td>0.96</td>
</tr>
</tbody>
</table>

The absence of enhancement of TPN reduction and oxygen evolution by isolated chloroplasts is difficult to explain if present ideas about the photosynthetic electron transport system are correct. Although an enhancement might possibly be observed after a more extensive search for optimal conditions it appears to be unlikely, since a wide range of conditions has been tried and, furthermore, enhancement was very readily and consistently observed with intact cells. It is noteworthy that the experiments on whole cells and on cell-free preparations were made with identical apparatus and illumination techniques.

At present, therefore, there appears to be some controversy about whether isolated chloroplasts show an Emerson effect. Mayne and Brown (Plant Physiol., 37, lxv, 1962) have also failed to demonstrate enhancement. Gordon (Plant Physiol., 38, 153, 1963) reported an enhancement effect on the photoreduction of TPN by extracts of the red alga Laurencia obtusa, but the rates he observed were extremely low. Govindjee, Govindjee, and Hoch (Biochem. Biophys. Res. Commun., 9, 222, 1962) showed a synergistic effect of white light and red light on TPN reduction, and Bishop and Whittingham (Nature, 197, 1225, 1963) have observed enhancement of the ferricyanide Hill reaction in flashing light. These positive results have been obtained under rather specialized conditions; their relation to a true Emerson effect may be tenuous and remains to be demonstrated experimentally. No one has yet found an enhancement with cell-free preparations comparable to that observed with intact cells. The only possibly positive evidence we have obtained for the existence of an Emerson effect in chloroplasts is that the action spectra for the rates of TPNH and ATP formation at saturating light intensities show a considerable degree of structure (Black, Fewson, Gibbs, and Gordon, to be published, J. Biol. Chem., 1963). McLeod (Year Book 59, p. 342) observed a similar phenomenon for oxygen evolution by intact cells of several algal species. French has suggested that this effect and the Emerson effect may have a common origin.

Probably the most significant results
on cell-free systems could be obtained by examining the enhancement of carbon dioxide fixation by isolated chloroplasts, since this is the system most closely allied to that existing in whole-cell photosynthesis. Unfortunately rates of chloroplast carbon dioxide fixation are so low (about 2 per cent of the rate for intact tissues) and inconsistent that it is doubtful whether such experiments would have much value at present.

**Action Spectra for Separated Light Reactions in Photosynthesis**

*David C. Fork*

The concept that photosynthesis requires two different light reactions originated largely with Emerson's experiments on the enhancement effect and with Blinks's experiments on chromatic transients. Since then considerable effort has been devoted to determining how different light reactions participate in photosynthesis.

Witt and co-workers have been able to study several of the intermediate reactions of photosynthesis by measuring specific spectral absorption changes caused by illumination. Furthermore, they noted that excitation of *Chlorella* by far-red light (~710 m\(\mu\)) resulted in absorption changes corresponding to oxidation of a special chlorophyll \(a\) and cytochrome (light reaction \(h_{\nu I}\)), whereas excitation of light reaction \(h_{\nu II}\) with shorter wavelengths (~670 m\(\mu\)) resulted in absorption changes corresponding to reduction of compound X, since identified as plastoquinone.

It was possible by appropriate treatment to obtain samples of chloroplast fragments which showed only one or the other of these light reactions at a time.

We were then able to measure the action spectrum for each separated light reaction. In the terminology of Witt's laboratory, reaction \(h_{\nu I}\) results from the activation of what we have called the long-wavelength chlorophyll reaction and is evidenced by a decrease in absorption at 433 and 703 m\(\mu\), whereas reaction \(h_{\nu II}\) results from activating the accessory pigment system and is evidenced by a decrease in absorption at 475 m\(\mu\) and an increase at 515 m\(\mu\). Since these reactions are interrelated in the chloroplasts, it is necessary to inactivate the one not under investigation to prevent complications caused by simultaneously occurring reactions.

Separation of light reaction \(h_{\nu I}\) and its action spectrum. Vernon, Kamen, and Zaugg demonstrated that aged chloroplasts which can no longer evolve oxygen can, nevertheless, reduce pyridine nucleotide in the light if appropriate reduced compounds are provided. Witt has found that aged chloroplasts with reduced \(n\)-methylphenazonium methosulfate (PMS) and excess sodium ascorbate exhibit changes of absorption upon illumination which correspond to oxidation of cytochrome and chlorophyll. With increased concentrations of reduced PMS, only the negative absorption changes at 433 and 703 m\(\mu\) are seen, indicating that reduced PMS couples directly to chlorophyll 703. Furthermore, the identical behavior of the 433- and the 703-m\(\mu\) absorption changes with different physical and chemical parameters indicates that they are caused by one and the same phenomenon—oxidation of a particular form of chlorophyll. The discovery of the 703-m\(\mu\) change was made by Kok, whose detailed study of this absorption change also indicates that it is related to oxidation of chlorophyll.

Aged chloroplast fragments that no longer exhibited changes of absorption at 515 m\(\mu\) (discussed later) were therefore used for the determination of the action spectrum for chlorophyll oxidation. In order to use red actinic light we measured
the absorption change at 433 μm instead of at 703 μm. That PMS, reduced by excess sodium ascorbate, was coupled to chlorophyll directly rather than through cytochrome was seen by the absence of absorption changes characteristic of cytochrome at 401 μm. At each actinic wavelength used for determining the action spectrum, the 433-μm absorption change was obtained as a function of light intensity. Figure 5 shows the absorption change at 433 μm for four wavelengths as a function of the incident light intensity. An intensity (100 × 10^{14} quanta cm^{-2} sec^{-1}) was chosen that fell within the linear region of all the light intensity curves. The resulting absorption changes were then plotted as the action spectrum in figure 7.

Separation of light reaction hνII and its action spectrum. Bishop has shown that Hill reaction activity can be restored by recondensing plastoquinone on chloroplasts previously extracted by petroleum ether. A parallel experiment by Müller and by Weikard in Witt's laboratory has demonstrated that this extraction abolishes the negative change of absorption upon illumination at 475 μm and the positive change at 515 μm and that recondensation of plastoquinone restores them. The absorption changes at 475 and 515 μm are thus found only in the presence of plastoquinone. The reduction of plastoquinone seems to be correlated with oxygen production (Müller et al., Proc. Royal Soc. London, B, 157, 313, 1963).

The observation, mentioned earlier, that plastoquinone reduction is more effectively mediated by 670 μm than by 710 μm was documented more quantitatively by measuring the action spectrum for the production of the 515-μm absorption change. For this purpose freshly prepared chloroplast fragments were used. The time course of the absorption change at 515 μm for fragments suspended only in tris buffer exhibited induction effects. A transient positive spike occurred upon illumination. A steady-state absorption change, lower than the initial spike, was
attained after about 1 second in the light. The height of the 515-μm absorption change spike was increased by a previous dark period. The oxygen-production spike of chloroplasts was likewise found to be increased by a dark period (Year Book 61, p. 336). With these chloroplast fragments the time required for the 515-μm absorption to decay to half of its original value (t_{1/2}) upon darkening was about 100 msec.

A similar preparation of chloroplast fragments in tris buffer but with added potassium ferricyanide and 2,6-dichlorophenol indophenol (DPIP) showed a profound alteration of the 515-μm absorption change, which no longer showed induction effects, was smaller, and had the t_{1/2} shortened to about 40 msec. These additions apparently rendered the long-wavelength chlorophyll reaction inoperative, since no absorption changes characteristic of this system (at 703 μm) were detected. Witt has suggested that ferricyanide functions by keeping the long-wavelength chlorophyll oxidized while DPIP serves as a trap to intercept electrons that normally are passed to long-wavelength chlorophyll a.

As was done for the 433-μm changes, measurements of the 515-μm absorption change were made as a function of increasing light intensity. Figure 6 shows the absorption change at 515 μm as a function of light intensity for four wavelengths. A light intensity (25 × 10^{14} quanta cm^{-2} sec^{-1}) was chosen such that, for each light intensity curve, it fell within the region where the 515-μm change was linear with intensity. The resulting 515-μm absorption changes were plotted as the action spectrum shown in figure 7. This spectrum has a peak at 674 μm, a shoulder at 650 μm, and a far-red limit around 705 μm. The action spectrum for the 433-μm absorption change, in comparison with that for the 515-μm change, remains higher at far-red wavelengths and has a limit around 737 μm. It shows a small shoulder around 650 μm, a peak at 682, and another shoulder around 697 μm.

---

**Fig. 6.** The 515-μm absorption change as a function of actinic light intensity for representative wavelengths at 625, 654, 674, and 697 μm. The solution contained 1.5 × 10^{-6} M DPIP, 1 × 10^{-4} M K_{3}Fe(CN)_{6}, 5 × 10^{-2} M tris buffer (pH 7.2), and chloroplast fragments which gave a final chlorophyll concentration of 0.115 mg per ml. The transmission of this suspension in the 1-mm-path-length cuvette at 674 μm was 22 per cent; temperature 21°C. A fresh sample was used for each saturation curve. The data from these curves and other measurements were used to plot the action spectrum of figure 7.
Fig. 7. The action spectrum for the 515-\(\mu\)m and 433-\(\mu\)m absorption changes obtained as described in the text. Monochromatic light to excite photosynthesis was obtained from a 1000-watt tungsten-filament lamp by filtering white light through 5 cm of water, 2 mm of a Schott OG-2 glass filter, and appropriate Schott interference filters having half-bandwidths from 11 to 15 \(\mu\)m.

The resolution of the absorption band of chlorophyll \(a\) by French and co-workers into three components at 673, 683, and 695 \(\mu\)m permits an understanding of the peaks and shoulders of these action spectra. It appears that chlorophyll \(a\) 674 and chlorophyll \(a\) 695 provide energy to drive light reaction \(h\nu_1\).

Oxygen evolution, if intimately involved with the 515-\(\mu\)m absorption change, would also be expected to continue in a separated system and to be sensitized by light absorbed by chlorophyll \(a\) 673 and chlorophyll \(b\). Oxygen evolution was, in fact, observed by Losada, Whatley, and Arnon in chloroplast fragments treated with DPIP-ferricyanide. Since DPIP-ferricyanide treatment also permitted separation of the light reaction responsible for the 515-\(\mu\)m absorption change, it may be inferred that Losada's chloroplast fragments and those used here were comparable. Losada found a peak in the action spectrum for oxygen evolution at 644 \(\mu\)m for these chloroplast fragments—a result qualitatively similar at least to the action spectrum for the production of the 515-\(\mu\)m absorption change.

Witt and co-workers have found that the 515-\(\mu\)m absorption change in \textit{Chlorella} is biphasic in that it starts with a rapid temperature-independent increase, which is followed by a further slow rise that is temperature dependent. As was mentioned earlier, the absorption change at 515-\(\mu\)m for chloroplast fragments suspended in tris buffer is also biphasic. Upon addition of DPIP and ferricyanide, however, the biphasic behavior disappears and only the rapid rise remains. The slow rise at 515 \(\mu\)m may be associated with absorption by long-wavelength chlorophyll components, since the action spectrum that we measured for the 515-\(\mu\)m absorption change in the unseparated system had a longer wavelength limit than it had in the separated system.
NATURE OF THE FORMS OF CHLOROPHYLL a

J. S. Brown

The concept that chlorophyll a exists in the plant in different forms is now generally accepted. Most of the evidence is consistent with the existence of two major forms absorbing at about 670 and 683 m\(\mu\), and a minor form, variable in amount, at about 695 m\(\mu\). Besides differing in absorption maxima, these forms function differently in photosynthesis.

Information about the physical-chemical state of these three forms is practically nil. In fact, we know very little about the chlorophyll lipoprotein complex as it exists in the plant.

By contrast with the situation in mature green plants, the nature of the pigment complex in etiolated plants is well known through the work of Dr. Smith. An active protochlorophyll lipoprotein particle has been isolated. It is a spheroid having a long axis of about 200 Å. When etiolated plants are first illuminated, the protochlorophyll is transformed to chlorophyll a with an absorption maximum near 684 m\(\mu\). After further illumination more chlorophyll is formed, and the absorption maximum shifts to about 675 m\(\mu\), the peak normally observed in mature leaves. However, after chlorophyll has accumulated, it has not been possible to isolate homogeneous suspensions of particles comparable to protochlorophyll holochrome.

Small fragments of spinach chloroplasts obtained by sonication still retain activity for the Hill reaction. Dr. Park at the University of California, Berkeley, has recently taken electron micrographs of these fragments which show them to be made of spheroids resembling the protochlorophyll particles.

Dr. Allen at the Kaiser Laboratory, Richmond, California, has prepared small particles from Chlorella by alternate freezing, grinding, sonication at —40°C, and finally a sucrose density gradient centrifugation. These particles with an absorption maximum at 672 m\(\mu\) will perform part of the photoreactions of the whole chloroplast, including a modified Hill reaction, and they give a rapid EPR signal. The function of chlorophyll a 672 as a part of the "accessory pigment system" is becoming firmly established, particularly through studies of action spectra for enhancement, which have clearly shown a peak at about 670 m\(\mu\).

The nature of the chlorophyll form absorbing at 685 m\(\mu\) is less well known. It may be either a discrete particle similar to C\(_a\)672, but with a different lipoprotein composition, or a particular structural arrangement of the chlorophyll lipoprotein complex, or perhaps a dimer of the pigment molecules.

Support for the suggestion that the C\(_a\)685 particle is a discrete entity comes from the study of aged Euglena chloroplasts. When such chloroplasts were broken into very small pieces and centrifuged at 144,700g, two fractions were obtained: one, a sediment having a complex "red" absorption band with a large proportion of the absorption attributable to a pigment, P\(_a\)710, with absorption maximum at 710 m\(\mu\); the other fraction, left in the supernatant, which had a simple "red" absorption band with a maximum at 685 m\(\mu\), C\(_a\)685. A comparable separation of the two fractions was obtained by centrifugation in a sucrose gradient. Since the fractionation has been done with only aged material, it is possible that the C\(_a\)685 particles are artifacts formed during the aging of the cells.

Evidence also supports the thesis that C\(_a\)685 is a particular structural arrangement of the chlorophyll lipoprotein complex. Previous reports have described how many deleterious agents such as heat, intense light, acids, and some detergents and fat solvents destroy the absorption at 685 m\(\mu\) more than at 670 m\(\mu\). Experiments demonstrating selective extraction with ether have been performed in the past year.
When aqueous pigment extracts from *Euglena* were treated with ether, a relative decrease in absorption at about 685 mµ was observed. With Swiss chard chloroplasts, this decrease was present but smaller. Figure 8A illustrates the types of absorption changes obtained by ether treatment of aqueous suspensions prepared from *Euglena* grown with intense light; figure 8B, from cytoplasmic fractions of *Euglena* aged to form P₇₁₀.

The observation that ethyl ether selectively extracts the longer-wavelength forms of chlorophyll *a* in a way that depends on various factors encourages us to continue these studies on the nature of the chlorophyll lipoprotein complex.

**New Algae for Photosynthesis Studies**

*J. S. Brown*

Comparative studies of action spectra and of the variation of enhancement effects with wavelength in algae with different pigment systems have provided the basis for the current concept of the need for two separate photochemical steps in photosynthesis. We are beginning a search for plants, particularly algae, with different photosynthetic pigments and with different ratios of the known pigments that may be particularly suitable for studies of the interaction of pigment systems.

An interesting green alga, resembling *Stichococcus* in appearance, has been isolated from a stock solution of 1 M MgSO₄·7H₂O. This organism grows well in a liquid or agar Knop's medium with 0.5 M MgSO₄ using 5 per cent CO₂ in air for the liquid cultures. Previous studies with known *Stichococci* have indicated that this genus can be adapted rather easily to a wide range of salt concentrations. The ratio of chlorophyll *a* to chlorophyll *b* in *Stichococcus* growing with high salt concentrations is less than 2 to 1. Most green plants have a ratio of about 3 to 1, and *Euglena* grown with very weak light has a ratio of 7 to 1.

Figure 9 compares derivative absorption spectra of *Stichococcus* and *Dunaliella* whole cells. The short-wavelength, positive, derivative peak is caused by chlorophyll *b* absorption. The maximum chlorophyll *a* absorption at about 680 mµ is where the curve changes from positive to negative. The shoulder on the positive portion of the chlorophyll *a* derivative peak is caused by the overlapping absorption bands of C₆670 and C₆685. The relative height of this shoulder with respect to the zero line indicates the
proportions of these two forms of chlorophyll $a$.

The spectrum for *Dunaliella* is typical of most green algae and higher plants. By comparison *Stichococcus* has a higher proportion of chlorophyll $b$, and the proportion of $C_a685$ to $C_a670$ is also significantly higher. This organism may be valuable for investigation of the nature of the chlorophyll forms and for study of the function and formation of chlorophyll $b$.

**PHOTOSYNTHESIS IN Stichococcus**

*Govindjee*

Measurements of action spectra of photosynthesis and comparisons between action and absorption spectra have been used for a long time in the evaluation of the role of different pigments in photosynthesis. Dr. Jeanette S. Brown has isolated and cultured a particular strain of a green alga, probably a *Stichococcus*, which contains a high proportion of chlorophyll $b$ as described in this report.

Rates of photosynthesis of this alga were measured by means of a platinum electrode as described elsewhere (*Year Book 60*, p. 362). The circulating medium (Knop's solution $+ 1 M \text{MgSO}_4$) was gassed with 5 per cent CO$_2$ in air, and its temperature was 22$^\circ$C. The action spectrum of oxygen evolution was measured by the automatic action spectrophotometer (*Year Book 58*, p. 323), using a constant number of incident quanta at different wavelengths.

Figure 10 shows a typical time course of oxygen evolution in *Stichococcus* with 670-mu light. Upon repeated illumination, the rate of photosynthesis reaches a steady state after about 2 minutes. If the cells were left in the dark for a period of 10 to 12 hours it took 1 to $1\frac{1}{2}$ hours to attain the steady state. In addition, an initial spike was clearly noticeable. The time course of oxygen exchange in darkness (after illumination) shows an immediate sharp drop followed by a slower decline (see arrow in fig. 10).

Gingras, Lemasson, and Fork have observed a clear positive peak in the time course curve of *Chlorella* after white light was turned off. When DCMU was added just before the light was turned off, oxygen evolution in light was strongly inhibited but the positive peak was relatively unaffected, showing that the peak was not due to oxygen evolution. It is therefore believed to be due to a transient decrease of cellular respiration. On the basis of these considerations it may be inferred that the observed kinetics in *Stichococcus* may be simply due to an interaction of two opposing effects of
light on oxygen uptake—an inhibition and a stimulation of oxygen uptake. A clear stimulation in oxygen uptake is noticed as the rate curve dips below the original dark rate. An inhibition effect of light during the illumination period has been observed by Hoch and co-workers in several algae.

Figure 11 shows the action spectrum of photosynthesis made on a thin suspension of *Stichococcus*. There are two peaks—one at 672 m\(\mu\) due to chlorophyll \(a\), and the other at 653 m\(\mu\) due to chlorophyll \(b\). The ratio of the relative height of chlorophyll \(b\) to chlorophyll \(a\) peaks, observed in the action spectrum of photosynthesis in this alga, is 0.94—a very high ratio in comparison with that found in other green algae. This higher activity observed in the chlorophyll \(b\) region is paralleled by the findings of Brown that *Stichococcus* contains a high proportion of chlorophyll \(b\).

The enhancement phenomenon was studied by using 650-m\(\mu\) and 710-m\(\mu\) light separately and in combination. As shown in Table 4, an enhancement of rate of photosynthesis ranging from 1.2 to 2.3 was obtained between the two wavelengths. The occurrence of enhancement indicates that two photoreactions are also necessary for *Stichococcus* photosynthesis.

The same culture that showed enhancement at one time failed to show it at another time under very similar conditions. A difference in the effect of light on respiration (during illumination) in separate and in combined lights may complicate the observed results. Unless the effects of light on respiration are very variable, however, such different results should not be found in enhancement studies. The factors responsible are under investigation. Why enhancement is not always observed is still an open question. Perhaps the occurrence of enhancement depends on the pool of an unknown compound, and a change in pool size (indirectly affected by respiration) may be the cause of variable results on enhancements.

Govindjee acknowledges the support from the National Science Foundation grant G19437 and a Carnegie Institution of Washington travel grant.

**TABLE 4.** Enhancement in *Stichococcus*, 22°C, 5 Per Cent Carbon Dioxide in Air

<table>
<thead>
<tr>
<th></th>
<th><strong>Rate of photosynthesis in arbitrary units</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><strong>710 m(\mu)</strong></td>
</tr>
<tr>
<td><strong>B</strong></td>
<td><strong>Combined Lights</strong></td>
</tr>
<tr>
<td>12.8</td>
<td>22.0</td>
</tr>
<tr>
<td>12.8</td>
<td>45.0</td>
</tr>
<tr>
<td>18.2</td>
<td>182.0</td>
</tr>
</tbody>
</table>

* The Emerson enhancement is defined here as \(E = (D - B)/A\).
SOME OBSERVATIONS ON THE REVERSIBLE LIGHT-INDUCED INHIBITION OF RESPIRATION IN NONSULFUR PURPLE BACTERIA

D. C. Fork and J. C. Goedheer

Nakamura, in 1937, was the first to report inhibition of respiration by light in a purple bacterium. Since then numerous investigators have confirmed the observation and have studied it in more detail. One interpretation is that substrate hydrogen, which normally reduces oxygen via the respiratory chain, is diverted upon illumination to the reduction of carbon dioxide through the photochemical apparatus. Nakamura, therefore, assumed that it was possible to study bacterial photosynthesis by measuring light-induced changes of respiration. Duysens in his thesis in 1952 (Stelling VI) also suggested that action spectra for photosynthesis in bacteria could be determined by measuring the inhibition of their respiration by light. We have measured the action spectrum for the inhibition of respiration by light, compared it with the spectral absorption by the photosynthetically active pigments, and also have observed the influence of a redox dye and of a poison of oxygen evolution on this light-induced inhibition.

Horio and co-workers (personal communication) have reported (J. Biol. Chem., in press) an action spectrum for light inhibition of respiration in *Rhodospirillum rubrum* in the 410- to 610-m\(\mu\) region which appears to be similar to those reported here.

The bacteria used in this study were grown anaerobically under incandescent illumination at about 25°C: *Rhodospirillum rubrum* in 1 per cent peptone and 0.5 per cent NaCl, pH 7; *Rhodopseudomonas spheroides* in 0.5 per cent yeast extract, 0.5 per cent MgSO\(_4\), 0.3 per cent L-malic acid, and 0.2 M phosphate buffer at pH 6.8. Tap water was used for both media. For the action spectra determinations the cells were harvested after 1 day’s growth.

The Teflon-covered platinum electrode described in *Year Book* 61, page 343, was used to follow respiratory changes. A drop of a thin suspension of bacteria in fresh medium was placed on the Teflon-covered electrode and held in place with another piece of 6-micron-thick Teflon film. Air was then passed at a constant rate over this suspension. The liquid-circulating system was not needed when the electrode was being used for action spectra determinations but was used when the effects of inhibitors were being studied.

Monochromatic light was obtained from a 500-mm-focal-length Bausch and Lomb monochromator with a 100 by 100 mm grating having 600 grooves per millimeter. Each action spectrum was done in three parts with the slits set to pass a beam having a half-bandwidth of 3.3 m\(\mu\): from 940 to 740 m\(\mu\), a 600-m\(\mu\) cutoff filter to remove spectral impurities from second-order wavelengths and a 48 per cent transmission neutral density filter were inserted in the monochromator beam; from 650 to 550 m\(\mu\), only the 48 per cent transmission filter was used; and from 550 to 450 m\(\mu\), no filters at all. The precision of measurement was lower for 550 to 450 m\(\mu\) than for the other portions of the action spectrum because the light intensities were low and the resulting responses small. The monochromator wavelength dial was turned manually 1 m\(\mu\) every 10 seconds, and a continuous recording of respiration was made. This record was then corrected for equal incident quanta and for loss of activity with time (if any) and replotted at 5-m\(\mu\) intervals.

A time course for the inhibition of respiration by 880-m\(\mu\) light is shown in figure 12. A deflection of the trace above the dark base line indicates decreased respiration, since more oxygen, diffusing
Fig. 12. The time course of inhibition of respiration of *Rhodospirillum rubrum* upon exposure to 880-μ light (497 ergs cm⁻² sec⁻¹) having a half-bandwidth of 10 μ. The cells were harvested after 2 days' growth and resuspended in fresh medium. Gas phase, 5 per cent CO₂ in air.

Fig. 13. Inhibition of respiration as a function of light intensity. For *Rhodopseudomonas* the 850-μ light used had a half-bandwidth of 3.3 μ. Cells from a 1-day-old culture were suspended in fresh medium and gassed with air. This sample was used for the determination of the action spectrum. For *Rhodospirillum* the 880-μ light used had a half-bandwidth of 10 μ. The 2-day-old culture was gassed with 5 per cent CO₂ in air. Another sample was used for the action spectrum in figure 15.
from the circulating medium, can be reduced at the electrode when the respiratory uptake is lowered. Oxygen production will cause a deflection in the same direction. However, Johnston and Brown showed in their study with the O^{18} isotope that the photosynthetic bacteria do not evolve oxygen. When the bacteria on the electrode are made anaerobic (gas phase N_2) the light effect disappears (unlike oxygen evolution in green plants, which may continue under anaerobic conditions). The potent inhibitor of oxygen evolution, DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, at a concentration of 6.5 \times 10^{-5} M did not have an appreciable effect on light-induced inhibition of respiration.

The time course shown in figure 12 indicates that the inhibition becomes constant after about 2 minutes in the light. Darkening the cells causes the recorder tracing to dip below the dark base line established previously. The respiration rate attains its former dark level after about 12 minutes. This temporarily increased respiration after a light exposure is apparently similar to the respiratory stimulation observed after the red alga Porphyridium cruentum was exposed to red light (Year Book 60, p. 352). Respiratory stimulation after exposure of Rhodopseudomonas to 850-\mu light has also been seen frequently, but not invariably.

The inhibition of respiration as a function of incident light intensity is shown in figure 13. Since these curves start to bend at low light intensity, the action spectra were determined with intensities as low as possible. The same Rhodopseudomonas sample was used to determine the saturation curve of figure 13 and also the action spectrum. At 850 \mu the intensity of the monochromator beam when used as described above was 64.3 ergs cm^{-2} sec^{-1}. At this intensity the effect per unit intensity is 16 per cent less than at the lowest usable intensities. Since the calculations of the action spectrum were made by assuming a linear relationship between inhibition of respiration and light intensity the action spectrum is somewhat flattened where this error occurs.

The action spectrum for light-induced inhibition of respiration in Rhodopseudomonas (fig. 14, lower curve) has peaks at 850, 800, 590, 510, and 480 \mu and a shoulder around 880 \mu. The absorption spectrum of chromatophores (upper curve), prepared from a different sample, is shown for comparison. The action spectrum for Rhodospirillum (fig. 15, lower curve) has peaks at 880, 810, 595, \sim520, and \sim485 \mu. The absorption spectrum of a chromatophore preparation from a different sample is also shown (upper curve).

The light inhibition of respiration was disrupted by the redox dye n-methylphenazonium methosulfate (PMS). Figure 16 shows this effect with Rhodospirillum. Exposure to 880 \mu before the addition of PMS resulted in a 75 per cent inhibition of respiration. (The zero respiration line is the electrode dark current after the cells were killed at the end of the experiment by adding formaldehyde solution in a final concentration of about 4 per cent to the circulating solution.) After a 4-minute exposure to 880 \mu the cells were darkened and a temporary stimulation of respiration about 25 per cent greater than the previous dark respiration occurred. PMS was added to the circulating solution while the cells were in the dark (arrow); an 80 per cent increase in the dark uptake of oxygen resulted. Another exposure to 880 \mu after the addition of PMS resulted in only an 18 per cent inhibition of respiration. The time course of inhibition of respiration upon illumination in the presence of PMS shows an induction period followed by a slow increase. The time course of the respiratory recovery in the dark after 880 \mu is complex. No stimulation of respiration followed illumination in the presence of PMS. Repeated exposures to 800 \mu light resulted in a gradual decrease in the amount of light-induced inhibition of respiration as well as a gradual retardation in the time course.
Fig. 14. Lower half: Action spectrum for inhibition of respiration in Rhodopseudomonas. Upper half: Absorption spectrum of chromatophores in phosphate buffer.
Fig. 15. Lower half: Action spectrum for inhibition of respiration by light in *Rhodospirillum rubrum*. One-day-old culture in growth medium; gas phase, air. Upper half: Absorption spectrum of chromatophores in phosphate buffer from a different sample from that used for the action spectrum.
The action spectra for light-induced inhibition of respiration follow the absorption of the photosynthetically active pigments and suggest an intimate coupling between photosynthesis and respiration. Horio and Kamen explained light-induced inhibition of respiration on the basis of a competition between the photoactive pigments and an intermediate in the respiratory electron-transport chain. Nishimura and Chance advanced a similar idea, that electron transport for both photosynthesis and respiration passes through a common cytochrome.

An explanation for the inhibition effect based on a competition between photophosphorylation and oxidative phosphorylation for a common phosphate acceptor was ruled out by Katoh, who found added adenosine diphosphate to be without effect. He also noted that inhibitors of photophosphorylation such as o-phenanthroline and 2,6-dichlorophenol indophenol did not affect photoinhibition.

PMS may act as a by-pass of an intermediate common to both photosynthesis and respiration, mediating a more rapid flow of electrons to oxygen, which would result in an increased dark oxygen uptake and a decreased effect of light on respiration.

The activity of carotenoids in relation to bacteriochlorophyll in sensitizing inhibition of respiration is higher in Rhodopseudomonas than in Rhodospirillum. Goedheer has also observed a more efficient transfer of energy for carotenoids to bacteriochlorophyll in Rhodopseudomonas than in Rhodospirillum.
Is the Photoconversion of Protochlorophyll Immediate?

Axel Madsen

Introduction. Since the transformation of protochlorophyll into chlorophyll \( a \) is dependent on temperature, it has been suggested by Smith and Benitez (Plant Physiol., 29, 135, 1954) and by Boardman (Biochim. Biophys. Acta, 64, 205, 1962) that some steps other than pure photochemical reactions are involved. These other reactions, if any, could possibly be slower than the photochemical step. They might be detected as a time delay in the transformation after exposing active protochlorophyll to sufficiently short illumination and then immediately measuring any changes observed in the absorption spectrum. Previous investigations have shown that, in chloroplasts from etiolated barley, the transformation of protochlorophyll is completed in less than 0.04 second after illumination (Madsen, Progress in photobiology, Proc. III Intern. Congr. Photobiol., p. 567, 1960). The aim of the present work was to make faster measurements of the transformation of protochlorophyll in intact leaves and in protochlorophyll-holochrome preparations.

Plant material. Barley was grown in darkness for 6–7 days at 26°C. About 2.5-cm-long pieces were cut 1 cm below the apex and were placed close together between two glass slides. Beans were grown under the same conditions (for 18 days), and the protochlorophyll holochrome was prepared according to Smith (Year Book 57, p. 287) and Smith and Coomber (Year Book 59, p. 325). All operations were carried out under a weak green light.

Measurements. The transformation was measured by the increase in absorption by chlorophyll of a weak beam at 680 mp.

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Fig. 17. Arrangement for measuring absorption of light in intact leaves. Shutter I between the sample and the phototube (RCA 5819) is closed during the flash; it opens for the measuring beam when shutter II has cut off the flash beam. The proper sequence, achieved by an adjustable microswitch and adjustable relays, is: (1) triggering the flash; (2) closing shutter II; (3) triggering the oscilloscope; and (4) opening shutter I.
immediately after a bright flash of light covering most of the visible spectrum.

The setup for measuring changes in absorption in leaves is pictured in figure 17. Light from a 6-volt tungsten lamp was passed through an interference filter (B-1 6800, Baird Atomic, Inc.) and focused on the leaves. Flashes of light were obtained from a photographic flash apparatus (Honeywell Stroboban Futuramic II Electronic Flash). To shorten the duration of illumination a shutter (a tin can with oppositely placed holes) was put between the flash apparatus and the leaves. The shutter was rotated 90° in its housing by means of a spring after being released by an electromagnet; as it moves with increasing speed it produces a very sharp cutoff. The closing time is about 1.5 msec, depending on the spring tension. Another shutter is placed between the leaves and the photomultiplier for protection against the high-intensity flash. In this shutter a spring-loaded disk sector is held by a trigger in a position where it closes the aperture in its housing. When the shutter is released by hand it actuates a microswitch and opens to the phototube. Its opening time is 1.5 msec. The moment of contact with the microswitch can be adjusted. By means of a battery and suitable wiring the microswitch releases the flash beam shutter and, through adjustable relays, triggers the flashtube and the oscilloscope. With proper adjustment of the microswitch and the relays it is possible to have the setup ready to measure changes in the absorption in the leaves 4 msec after the beginning of illumination.

In the arrangement for measuring transformation in holochrome preparations the flash beam is oriented perpendicular to the measuring beam, figure 18. The sample is held in a cell made of Perspex mounted in a black holder provided with two perpendicular perforations, for the flash beam and the measuring beam, respectively. To get rid of stray light the measuring beam is focused on a narrow aperture 20 cm in front of the phototube. The shutter

![Diagram](image)

**Fig. 18.** Arrangement for measuring absorption of light in solutions. The incident flash beam is normal to the sample and perpendicular to the measuring beam. Instead of a shutter, a 20-cm-long nonreflecting tube is mounted in front of the phototube to protect it. A switch triggers the flashtube, the oscilloscope, and the shutter movement. The sequence of events is determined by adjustable relays.
between the flash tube and the sample is the same as in the setup shown in figure 17. The hand-operated switch releases the shutter and, by adjustable relays, triggers the flash tube and the oscilloscope.

Results with intact leaves. With the shutter between the leaves and the phototube in open position the absorption in the etiolated leaves before illumination was marked on the scale of the oscilloscope. The shutter was closed and then released by hand, starting the sequence of flash, shutter, and sweep. After a 1-msec illumination, which caused about 60 per cent transformation of the protochlorophyll, the oscilloscope showed a deflection of approximately 3 cm from the base-line level, which represented the absorption before illumination.

Any delay in the change of absorption after illumination would show up on the screen as a curve starting at the initial level and rising gradually to the level of absorption caused by illumination. This was not the pattern. Three milliseconds after illumination the track was horizontal, showing that in etiolated leaves the change in absorption is completed within 4 msec after the beginning of illumination. These experiments were carried out at room temperature.

If the temperature coefficient of the transformation results from reactions of enzymatic nature a possible delay of the change in absorption would be amplified by low temperature. Experiments with leaves stored in dry ice for 1 hour and measured when frozen showed that the rate of transformation was not affected measurably by low temperature. Only the amount of protochlorophyll transformed per flash was diminished. Measurements of the change in absorption during thawing after illumination showed that no increase in absorption followed the rise in temperature. The absorption was constant until the ice in the leaves disappeared. At this point sap leaked from the leaves and the absorption decreased rapidly.

Results with a purified protochlorophyll-holochrome preparation. When a preparation of protochlorophyll holochrome was used for the measurements, the amount of scattered light from the sample was very low and the setup shown in figure 18 made it possible to measure changes in absorption immediately after 1 msec of illumination.

The lower time limit for measurements in holochrome preparations is determined by the speed and the intensity of the flash. In these experiments 1-msec illumination was necessary to produce about 60 per cent transformation. Experiments carried out at room temperature showed that there is no further change in the absorption in protochlorophyll holochrome after 1-msec illumination. Ultra-short flashes of correspondingly higher intensity could be used for measuring the rate of transformation in holochrome preparations containing enough active protochlorophyll and no light-scattering material.

Summary. The formation of chlorophyll from protochlorophyll in intact etiolated leaves measured as the increase in absorption at 680 m\(\mu\) is completed within 4 msec after the beginning of illumination. Lowering the temperature did not slow the rate measurably. In protochlorophyll-holochrome preparations the change in absorption was completed during 1-msec illumination.

Fractionation of Protochlorophyll Holochrome by Density Gradient Centrifugation

Axel Madsen

Preparations of protochlorophyll holochrome often show very strong light absorption at wavelengths shorter than 460 m\(\mu\). The sharp absorption maximum of protochlorophyll holochrome at 442 m\(\mu\) is thus obscured. Furthermore, the preparations often contain light-scattering components that show up in the absorption spectra as an apparent increase in absorption toward shorter wavelengths.
These disturbing factors increase the difficulty of making exact measurements of the transformation of protochlorophyll holochrome to chlorophyll holochrome. An attempt was therefore made to purify the material further by centrifugation in a density gradient. A protochlorophyll-holochrome preparation was made from dark-grown bean leaves according to Smith and Coomber (Year Book 59, p. 325).

The absorption spectrum of the preparation measured with a Beckman DK spectrophotometer, using a 1-cm cell, is shown in figure 19. The continuous line represents the absorption before illumination and the dashed line after 3 minutes' illumination with incandescent light. The peaks at 640 and 674 μm representing protochlorophyll holochrome and chlorophyll holochrome, respectively, indicate that the preparation contains active protochlorophyll holochrome, but the maximum at 442 μm is completely obscured.

One milliliter of this preparation was placed in a centrifuge tube on top of 4 ml of a density gradient solution made from sucrose in 0.1 M NaCl (density 1.07 to 1.20). It was spun at 96,000g for 5 hours in a Spinco "model L" ultracentrifuge. After centrifugation the solution was removed in 0.5-ml fractions from the bottom of the tube.

A device was built for the separation of these protochlorophyll-holochrome preparations after density gradient centrifugation, which appears to have general use in biochemical work. It is illustrated in figure 20.

The tube containing the centrifuged material is placed on the right in a frame
along with the tube to receive the desired fraction on the left as shown in the illustration. The frame is supported by a rack and pinion drive not shown in the figure. With the eccentric clamp compressing the micro tubing on the left side, measured samples are drawn up into the syringe. A stop allows fractions of equal volume to be withdrawn. Then with the eccentric clamp in its other position the sample is ejected into the left tube.

Figure 21 shows the absorption spectra of fractions 2 to 6, the numbers corresponding to positions in the tube shown in the insert.

The ratio OD$_{674}$/OD$_{640}$ gives a measure of the activity of the protochlorophyll holochrome; the ratio OD$_{640}$/OD$_{440}$ is the criterion for purity of the preparation.

From top to bottom the fractions show decreasing contamination and increasing activity.

Fraction 1 contained only a trace of protochlorophyll holochrome and no yellow pigments, whereas fractions 7 and 8 absorbed strongly at wavelengths below 450 μm and contained a small amount of inactive protochlorophyll holochrome.

**Oxygen Exchange by Euglena Cells Undergoing Chloroplast Development**

*Jerome A. Schiff*

Dark-grown cells of *Euglena gracilis* var. *bacillaris* contain proplastids that develop into chloroplasts when the cells are exposed to light (Epstein and Schiff, *J. Protozool.*, 8, 427-432, 1961). The entire process of development, which takes 72 hours at the optimal intensity of light (100 foot-candles) can be studied in cells suspended in a resting medium lacking carbon and nitrogen sources so that the cells do not divide. Under these conditions electron microscopy of a thin section of cells shows that the first lamellar disc invaginates from the inner proplastid membrane at about 2 hours of development, and many more discs are produced subsequently. At about 12 hours of development the discs become extensively fused in twos and threes to form lamellae, although the first signs of fusion can be detected as early as 4 to 6 hours. From 12 hours onward the formation of lamellae is linear with time until the chloroplast is completed at 72 to 95 hours.

Previous studies at Brandeis University of the photosynthetic ability of *Euglena* cells while developing chloroplasts have shown that chlorophyll and carotenoid synthesis begins at a slow rate when the cells are exposed to light. The rate increases at about 10 to 12 hours and becomes linear with time (like lamellar
formation) until the chloroplast development is completed. Photosynthetic carbon dioxide fixation begins after about 4 to 5 hours of development and parallels the formation of pigments. Photosynthetic oxygen evolution has been followed as early in development as the precision of Warburg manometry permitted. Oxygen evolution was found to parallel the kinetics of chlorophyll formation from about 8 hours onward. It was of interest, therefore, to study oxygen evolution with a more sensitive method during the still earlier stages of chloroplast development to find out when photosynthetic oxygen evolution begins.

To do this the modification of the Blinks oxygen electrode devised by Fork

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**Fig. 22.** The oxygen exchange of dark-grown *Euglena* after being placed in 100-ic white light. The light was turned off for short times at various intervals. Each line is the continuation of the one above it. At first the light increases oxygen uptake. After 4 to 5 hours the photosynthetic system has developed, and from then on light produces oxygen. The time in hours marked on the curves is the total since the start of illumination.
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(Year Book 61, pp. 343-345) was employed. Dark-grown resting cells of Euglena were prepared as described previously (Stern, Schiff, and Epstein, Plant Physiol., in press) and centrifuged into a pellet. The packed cells were placed on the Teflon-covered electrode and held in place with a dialysis membrane. Resting medium, as described by Stern, Schiff, and Epstein, bubbled with 5 per cent carbon dioxide in air, was circulated at room temperature over the dialysis membrane. The above operations were performed under a green safelight. After the recorder trace showed that the rate of oxygen exchange had become constant, white light of 100 fc was turned on the cells at a time that was taken as zero on the record. To judge the extent of light-catalyzed changes in the rate of oxygen exchange, the light was turned off at half-hourly intervals for a long enough period to establish a dark base line (1 or 2 minutes, usually) and was then turned on to allow chloroplast development to continue. A negative deflection when the light was turned on indicated the occurrence of light-catalyzed oxygen consumption, whereas a positive deflection showed the rate of light-catalyzed oxygen evolution.

As can be seen in figure 22, illumination of the dark-grown cells results in a large increase in the rate of oxygen consumption, most of which is irreversible, as is shown by the changes recorded when the light was turned off and on 12 minutes after light exposure. A part, however, is reversible and constitutes a photostimulation of oxygen consumption. This photostimulation decreases with time of development until by 3.5 hours it has disappeared completely. At that time there is no change in rate of oxygen exchange when the light is turned off and on. Subsequently (4 hours and beyond) there is a net photostimulation of oxygen evolution the magnitude of which increases steadily with time as the chloroplasts develop.

In some experiments a somewhat different pattern was found. In figure 23 there is still the initial large irreversible increase in the rate of oxygen consumption, but it also shows several transients superimposed on the curve. When the light is subsequently turned off and on, complex transients accompany the changes. Each time up to 3.5 hours that the light is turned off the rate of oxygen exchange decreases but does not remain lowered. Instead it rises almost to the light level while still in the dark. Establishment of a true dark base line begins at about 3.5 to 4 hours, showing a true increase in oxygen evolution when the light is turned on. This is consistent with the time of occurrence of net oxygen evolution inferred from the data of figure 22. The conditions governing the differences between the two types of results (figures 22 and 23) are not known. Since the deflections measured in the second experiment are so much larger than the first, it may be that more cells were placed on the electrode and that interactions between cells led to the observed transients.

At any rate, two interesting findings come out of these experiments. First, there is a large irreversible increase in the rate of respiration when the dark-grown cells are initially induced to form chloroplasts by exposure to light. Second, the time for development of net photosynthetic oxygen evolution is about 4 hours for the experiment of figure 22 and about 1 hour for a transient oxygen evolution in figure 23 which becomes stable after about 3.5 hours.

The initial increase in respiration is probably correlated with the activation of synthetic pathways for the synthesis of chloroplast constituents. Chloroplast development in Euglena involves the synthesis of unique proteins not present in the dark-grown cells, including chloroplast cytochromes as well as photosynthetic pigments and other compounds. Before the cells become photosynthetically competent, the energy for these syntheses must come from respiration.

The appearance of net photosynthetic
oxygen evolution at about 4 hours of development correlates well with the inception of carbon dioxide fixation (4 to 5 hours of development). It is not known whether oxygen evolution precedes carbon dioxide fixation as reported by Smith (Plant Physiol., 29, 143-148, 1954) for higher plants. The onset of photosynthesis in Euglena occurs when only one or two unfused lamellar discs are seen by electron microscopy; in some cases these have fused to form the first lamella. This would suggest that the minimum structure necessary for photosynthesis is either the young lamella (consisting of a fused disc) or perhaps only a portion of the lamella—a single disc.

Acknowledgments. Some of the materials used in this work were provided by research grant RG-6344 from the National Institutes of Health to H. T. Epstein and J. A. Schiff, Biology Department, Brandeis University. The present project grew out of previous work done in collaboration with Dr. Epstein.

During the course of this work J. A. Schiff profited greatly from many discussions with Dr. James H. C. Smith about the physiology of chloroplast development.

Fig. 23. An experiment similar to that of figure 22 but with an earlier development of photosynthetic capacity, showing first as a short period of oxygen evolution that soon turns into oxygen consumption.
A Reversible Phosphate Electrode and Its Possible Application to Physiological Processes

James H. C. Smith

Phosphate metabolism is involved in photosynthesis, and evidence has been provided by Roux and his collaborators (Compt. rend., 251, 1925–1927, 1960) that the oxygen evolved in photosynthesis originates in phosphate. In order to examine the possibility of a correlation between oxygen evolution and phosphate absorption we intend to follow concurrently oxygen production and phosphate uptake under various conditions of temperature, light intensity, and light quality. An electrical procedure for simultaneously determining and continuously recording both oxygen evolution and phosphate uptake would be advantageous for this purpose. An apparatus already exists for tracing the evolution of oxygen under the desired conditions, and we hope that a similar arrangement can be developed for following phosphate uptake.

In Chance's laboratory an electrical method has been developed for estimating phosphate uptake by recording extremely small changes in hydrogen-ion concentration by means of a glass electrode. Since these changes would be difficult to distinguish from changes caused concurrently by gaseous exchange of carbon dioxide in photosynthesis or respiration, a different type of phosphate-ion detector is being sought.

Another type of electrode that responds to phosphate-ion concentration is the metal/metal phosphate/phosphate-ion electrode. Several such electrodes exist, for example: mercury/mercurous phosphate, lead/lead phosphate, zinc/zinc phosphate, and silver/silver phosphate. Of these, the last is the most easily handled, and its properties have been examined.

The silver/silver phosphate electrode has certain advantages over the others mentioned: (1) It is not fouled by reacting with the oxygen of the air or with water. The surface remains active for long periods of time. (2) Silver phosphate of the empirical formula Ag₃PO₄ is easily precipitated from solutions of a wide range of hydrogen-ion concentrations; it is easily washed free of extraneous ions. (3) The solubility of silver phosphate is relatively low. (4) The solubility equilibrium with phosphate approaches completion quickly.

Silver phosphate has the disadvantage of being rapidly decomposed by daylight, but, by working in relatively weak light from an incandescent lamp, the decomposition can be controlled so as not to pose serious difficulties.

The electromotive force cell used for measuring the phosphate-ion concentration was essentially a silver/silver-ion concentration cell. The reference electrode was a silver rod that dipped into a solution of constant silver-ion concentration provided by a saturated solution of silver phosphate in 0.05 M potassium borate buffer at pH about 8.8. The analytical electrode was a silver rod coated with a dense suspension of silver phosphate in agar gel which dipped into the same buffer solution but in which the silver-ion concentration was varied through variation of the phosphate-ion concentration.

By algebraic combination of the mathematical expressions for the ion-product constant of secondary phosphate ion, the solubility product for silver phosphate, and the Nernst equation for electromotive force, the following equation has been derived for the dependence of electromotive force on secondary phosphate-ion concentration:

\[ E = E_0 + 0.0195 \log [\text{HPO}_4^{2-}] \] (1)

That this equation holds for the experimental observations is shown in figure 24. The observed values of potential were taken from tracings made with a recording potentiometer. Their conformity with theoretical expectation amply
demonstrates the possibility of recording phosphate changes in a medium by electrical means.

In figure 24 the differences in electromotive force between the analytical silver/silver phosphate electrode and the reference electrode are plotted against the logarithms of the total phosphate concentrations in the analytical electrode compartment. The experimental points are marked with circles. The average of these points is designated by a cross through which a line of theoretical slope 19.5 (equation 1) has been drawn. The probable deviation of an experimental point from the theoretical was ±0.1 millivolt. The maximum deviation observed was —0.22 mv. With the probable deviation of 0.1 mv, the limit of sensitivity of the method is roughly 7 × 10⁻⁷ mole per liter. Since 1 ml is ample for a determination, this corresponds to 7 × 10⁻¹⁰ mole of phosphate.

The tracings from which the observed points were taken were produced by a recording potentiometer manufactured by the Nesco Instrument Company, Costa Mesa, California. Full-scale deflection of the potentiometer was 10 mv. For higher voltages, compensation was made by a Leeds and Northrup potentiometer.

The total phosphate concentration always included the phosphate contributed by the saturated silver phosphate. The value used, 0.000057 M/l, was estimated from the electrical measurements themselves. This solubility is greater than the 0.0000155 M/l given in the literature, probably because of the influence of the salt effect and pH of the buffer solution.

The silver phosphate electrode should be useful for phosphate determinations under many circumstances. It was found, however, in collaboration with Dr. D. C. Fork, that this electrode is impractical for certain biological applications, as, for example, in the measurement of phosphate uptake during photophosphorylation by broken chloroplasts. The medium used for this reaction contains chloride ion, ascorbate, and tris buffer, all of which react with silver ion. Interference by these constituents completely invalidates the use of the silver phosphate electrode. If a reaction mixture can be found that will permit action by the chloroplast fragments and yet not react with the silver phosphate, this electrode would have great advantages.

Work is in progress either to discover a medium in which the silver phosphate electrode can be used or to develop another electrode of comparable accuracy and sensitivity for measuring phosphate uptake in a medium suitable for biological systems.
Automatic Control of Scanning Speed for the Action Spectrophotometer

Jerome A. Schiff

The operation of the action spectrophotometer which continuously records the rate of photosynthetic oxygen evolution as a function of wavelength of the visible spectrum was described in *Year Book* 58, page 323. As the wavelength of light applied to the algal cells changes, the device records the effectiveness of each wavelength in mediating photosynthesis. In one mode of operation the measured rate of photosynthesis is used to control the light intensity so as to keep the photosynthetic rate constant. The reciprocal of the required intensity in quanta per unit of time is continuously plotted against wavelength.

On steep slopes of the action spectrum the light intensity must be changed rapidly or the scanning speed must be lowered to stay within the capacity of the system to adjust itself.

Since the action spectrophotometer is a servo device, there are certain limitations on its time of response which the scanning rate must not exceed. The characteristics of the algal cells themselves form a part of the servo loop controlling the light intensity. The response of the photosynthetic rate to a change of light intensity is not instantaneous. The lag period of photosynthesis is the limiting factor in the speed of response of the system. It is possible to scan at such a speed that the response time of the system is not exceeded during the periods of most rapid change in rate of oxygen evolution. However, a disadvantage is that more time is needed to complete an action spectrum, and the biological system under study may change its properties with time.

To avoid such delay, the rate of scan has been continuously adjusted by the investigator to suit the rate of change of oxygen evolution with wavelength. The constant attention of the investigator is required over the entire period, and often his reflexes are not fast enough to make the necessary compensation. To eliminate these difficulties, we have incorporated into the action spectrophotometer a circuit that permits the instrument to adjust its own rate of scan for optimal results. Such an arrangement is found in some commercial recording spectrophotometers.

A measure of whether the response time of the system is being exceeded is the degree of imbalance in the servo system. Ideally the servo should be in balance at every instant except for the small error voltage needed to activate it. The rate of scan should be such that at each increment of wavelength change the light intensity is in balance with the rate of photosynthesis before proceeding to the next wavelength increment.

The voltage delivered to the motor that adjusts the light intensity has therefore been used to reduce the wavelength sweep speed whenever it becomes excessive. The motor control voltage is rectified, filtered, and applied to the input of a "Labac" voltage controller, which contains silicon-controlled rectifiers operated by a magnetic amplifier. Its output drives the wavelength sweep. When the servo controlling the light intensity is balanced, its output is zero and no d-c signal appears in the control circuit of the magnetic amplifier. Under these conditions the monochromator motor scans at the maximum speed which has been set by the investigator at the beginning of the run. If, as the wavelength changes, the response time of the system is exceeded, a d-c voltage proportional to the imbalance appears in the control circuit of the magnetic amplifier which reduces the voltage to the monochromator's wavelength sweep motor circuit, thus slowing the rate of scan. If the imbalance is sufficiently great, the monochromator motor stops completely and waits until balance is restored before proceeding. In this way the optimal rate of scan is maintained. The automatic
scanning device requires a stable oxygen electrode and associated servo electronics, and also well behaved cells, since any small disturbances in the servo balance from these sources will slow or halt the scan.

AN AUTOMATIC PLOTTER FOR PLANE TABLE SURVEYING WITH A RANGE FINDER

C. S. French and R. W. Hart

The production of topographic maps from air photographs, though widely practiced, seems unlikely to displace plane table surveying for small areas of a few acres, particularly in heavily wooded regions. We have made an experimental model of an instrument that may facilitate plane table surveying.

Instruments built on the principle described here might be of practical value in topographic surveys for construction work ranging in scale from single houses to highways, for general grading purposes, and for filling in topography and detail on a base map of large scale. Other obvious applications are for archeological and ecological field surveys where an abundance of detail must be plotted. Such an instrument, however, being based on an optical range finder, does not seem applicable to high-precision work or small-scale mapping of large areas. One man could operate it where the objects or ground points to be plotted can be seen from the stations occupied, although a rod man would be essential for locating points on the ground in heavy brush.

The direction of points to be located on a map are easily obtained from an alidade on the plane table station; but the process of determining the horizontal distance to a point by tape or stadia measurements, reducing the data to the horizontal distance, and then scaling the data on the map takes some time for each point.

The machine we have made locates an index marker over the map position of a sighted point. This is done as rapidly as the telescope can be brought to bear on the point and a range finder adjustment made to bring two images in the telescope into coincidence. Also at this time the elevation of the sighted point appears on a dial reading directly in feet above or below a desired standard elevation. No calculations are needed.

An experimental model, cumbersome but usable, has been constructed; it is being tested to evaluate the practical utility of such a device and to estimate the precision attainable. The design of a production model has not been attempted. The principles, the construction of the experimental model, and some preliminary performance tests are described.

Principle. Our idea was to build a range finder into a telescopic alidade so that the setting crank of the range finder would simultaneously position an index over the map position of the sighted point. To use this principle for other than horizontal work a triangle solver of some sort must be included to convert the slope distance measured by the range finder into the true horizontal distance on the map and to calculate the elevation of the observed point.

This triangle solver receives two inputs: the slope distance, that is, a displacement proportional to the hypotenuse of the right triangle to be resolved; and the elevation angle from the instrument to the sighted object. The two output quantities of the resolver are proportional to the vertical and the horizontal sides of this triangle. The horizontal output is used to position the index marker on the map; the vertical output gives the elevation of the object on a dial.

General arrangement of parts. Figure 25 shows the relation of the instrument to the landscape. The round 32-inch-diameter plane table is mounted on a heavy tripod and arranged so that it can be leveled and rotated about its center point to align the map with the landscape. The table is then locked in position. The tripod and mounting is a war surplus "Instrument, observation, M1," from which the elbow telescope was removed.
Although the weight of the plane table is supported by a bearing on the rotating part of the original instrument, the table may be locked to a center rod attached directly to the fixed tripod head.

All other parts of the device are supported by the rotatable outer case of the original instrument mounting. This is of massive construction and has an azimuth scale readable to 0.1 mil. The scale is not
an essential part of the instrument but can be used if angular measurements of greater than graphical accuracy are desired. An angle iron frame supports the resolver in its cast aluminum case above the plane table and also carries an aluminum guideway with a slider. The slider positions the index marker over the map.

The telescope with its range finder components is mounted directly on one horizontal input shaft of the resolver. Since the optical axis of the telescope has to rotate about the center point of the plane table the resolver has to be off center. A counterweight under the table on the opposite side of the frame balances the weight of the resolver.

The table and also the entire assembly above it are removable for transporting the device from one observing station to the next. Adjustments are provided to allow crossed levels on the resolver to be made horizontal. A separate level is attached to the telescope.

The range finder. The resolver must receive an input proportional to distance. To allow direct coupling of the range finder and the resolver the range finder must have a linear scale. Standard varieties of range finders have inverse scales which would require a correction cam to link them to the resolver input. We therefore used a very simple range finder principle having a setting directly proportional to distance. It violates most of the accepted principles of good range finder design and requires precise guideways even for moderate performance.

The optical system of the range finder is shown in figure 26. One image of the object, O, is seen on the telescope cross hairs directly through the beam splitter. Another image coming from the mirror M, and reflected from the surface of the beam splitter, is coincident with the direct image for a certain mirror-to-beam-splitter distance MP. If the angle between the mirror and the beam splitter remains constant, the distance MP is proportional to OP, the distance from the instrument to the object. The mirror is moved on guideways parallel to the line MP by the range setting crank, which is also geared to the resolver’s hypotenuse input shaft. The map scale is determined by the angle between the mirror and the face of the beam splitter. The mirror angle is adjustable by three screws. The image seen straight through the dichroic beam splitter is orange; that reflected from its face is blue. At coincidence the image has its normal color.

The entire optical assembly and the guideways for the mirror are attached to the angle input shaft of the resolver as shown in figure 27. This shaft rotates in elevation about point P, the optical center of the instrument. Point P is located over the center of rotation of the plane table. The mirror is held on a carriage which runs on ball bearings between two aluminum guide tubes. A floating nut engages the screw without transmitting wobble of the screw to the carriage. The guide tubes and the drive screw are held in position at the top and bottom by aluminum castings. The limiting factor in the accuracy of the range finder measurement is the precision of the guide tubes.

At the suggestion of Dr. Ira Bowen we have tried out a movable two-mirror system in place of the single mirror. This arrangement, similar to a penta prism, gives a line of sight at nearly 90° which is independent of small errors in the carriageways. The scale is adjustable by setting the angle between these two mirrors. Because this system inverts the
Fig. 27. The range finder is mounted on the resolver's hypotenuse input shaft, which rotates on the axis $AB$. The horizontal output shaft of the resolver drives the index marker to the map position of the sighted object; the vertical output sets an elevation dial.
image a Pechan prism is placed in front of the beam splitter. Preliminary tests with this system show an improvement in repeatability of settings over the single-mirror system.

The resolver. The surplus mechanical resolver used for military fire control purposes is well suited for the present purpose because of its two concentric input shafts. It therefore serves as a stable mounting for the optical system as well as functioning as a computer. Unfortunately it weighs 23 pounds.

The outputs of the resolver are shafts which make about 1.6 revolutions for full scale. An aluminum drum was attached to the horizontal output shaft. A cable around it drives the index marker over the map. The path of the index is kept parallel to the optical axis of the telescope by 16-inch aluminum guideways.

The elevation output shaft carries a coarse dial reading directly in feet for several different map scales; a fine dial geared up 10:1 from the coarse dial facilitates the elevation reading. The zero positions of the dials are adjustable so that the reading can be made to refer to elevations above or below any desired elevation. Only one dial is shown in the figures.

Figure 27 shows how the optical parts are mounted on the resolver. The aluminum block $F$ clamps on the angle input shaft of the resolver and supports the telescope, the beam splitter $M_1$, and the assembly carrying movable mirror $M_2$. The mirror drive screw $D$ positions the mirror carriage on its guide tubes, which are supported by a casting attached to block $F$. The figure does not show the guide tubes, their supports, or the carriage.

The entire range finder assembly rotates around the $AB$ axis under the control of a worm gear and crank mounted on top of the resolver (omitted from the figure).

The double gear $G$ is carried on the resolver's hypotenuse input shaft concentric with the axis $AB$. The inner part of gear $G$ is a bevel gear driving the mirror-positioning shaft $D$; its outer part is a spur gear driven by a gear on the setting crank of the range finder.

Map scales. The scale of the map can be set for any usable range by changing the tilt of the mirror. To make the elevation dials read directly in feet without a conversion factor, however, the map scale and the elevation dial scales must agree. The dials have been graduated for the following scales: 1 inch = 10, 20, and 50 feet.

The relation between the elevation dials and the map scale is determined by the diameter of the drum on the horizontal output shaft of the resolver. This diameter, 3.155 inches, was chosen to give 1 revolution of the coarse elevation dial for 100 feet of elevation at a map scale of 1 inch = 10 feet. The fine elevation dial has 100 divisions, and, being $5\frac{3}{8}$ inches in diameter, can be read to 0.1 division. One division corresponds to 0.1-foot elevation at a map scale of 1 inch = 10 feet or to 0.5-foot elevation for a scale of 1 inch = 50 feet.

Performance. A line of test stakes at 20-700 feet is used for checking the accuracy of the distance measurement. At a scale of 1 inch = 10 feet the range of the instrument is 160 feet. Our best set of test data with that scale gave results within 1 foot. Limited tests on the elevation dials show an accuracy within 0.1 foot for small angles. Modifications are still in progress.

**EXPERIMENTAL TAXONOMY INVESTIGATIONS**

Progress has been made during the year along all the four approaches of our program designed to clarify mechanisms of natural selection and evolution of higher plants: combined genetic and transplant studies; controlled environment studies; quantitative physiological measurements; and work with tissue
cultures. The principles applied in pursuing these four approaches, all of which are focused on our major objectives, were reviewed last year (Year Book 61, pp. 311–312).

Emphasis in the present report is placed on a review of some of the accumulated results stemming from the first of these methods of inquiry. The most prominent feature is new evidence that establishes experimentally the general validity of an important concept—the principle of genetic coherence—as a reality in the evolution of higher plants. This principle is of importance not only for understanding processes of speciation but also for the orientation of the lines of inquiry aimed more specifically at discovering mechanisms underlying natural selection by the other three approaches.

GENETIC COHERENCE IN THREE SPECIES-COMPLEXES

William M. Hiesey, Malcolm A. Nobs, and Harold W. Milner

Genetic coherence is the tendency for characters distinguishing recognizable biological entities to be inherited together as a unit rather than to be distributed to progeny purely at random. Coherence can be studied experimentally by crossing contrasting ecological races of the same or closely related species and analyzing the characters of their first-, second-, and later-generation progeny. The expression of coherence tends to be overshadowed by the spectacular recombinations of characters typically found among F₂ progeny of such crosses.

Although the recombinations themselves are of much evolutionary interest, they tend to obscure and render difficult the experimental demonstration of genetic coherence. The study of coherence and of its consequences is greatly aided by observing the expression of characters in cloned F₁ and F₂ progenies grown at the altitudinal transplant stations at Stanford, Mather, and Timberline. A wide range of expression of the parents and of the progeny can thus be analyzed critically.

Evidence clearly demonstrating the existence of coherence in higher plants was found in the *Potentilla glandulosa* complex and was reported in Carnegie Institution of Washington Publication 615, volume IV of Experimental Studies on the Nature of Species, by Clausen and Hiesey. This complex is a highly diverse diploid group with \( n = 7 \) chromosomes. Distinguishing features marking contrasting ecological races are not transmitted by simple Mendelian inheritance but by systems of multiple genes whose components are carried in more than one chromosome. Combinations of dissimilar characters distinguishing one climatic race from another are thus carried by all the seven chromosomes and are loosely tied together through genetic linkage of the multiple gene systems.

The over-all result is that, when distinct races are crossed, there is a higher frequency among F₂ progeny of plants having characters like one parent or the other than would be expected on the basis of free random recombination. The extent of this tendency can be measured statistically by determining the value of the correlation coefficient \( r \) computed from the frequency with which any pair of dissimilar characters is expressed together in individuals of the same F₂ segregating population.

Since the expression of a given character in a particular environment is almost without exception the result of the interaction of multiple genes, it can be rated on an arbitrary scale covering the extremes of its expression. Coded on a scale of 9, the data for each such character for each individual F₂ plant of a population can be transferred to punched cards and then analyzed with the help of computing machines. The existence of genetic coherence in *Potentilla* was established by computing the values of the correlation coefficients between all possible combinations of 12 and 14 pairs of dissimilar characters in two highly seg-
regating F₂ progenies from crosses between contrasting altitudinal races. Comparable studies have been completed recently in *Achillea*, a genus of the sunflower family, and *Mimulus* of the figwort family. The results from both groups are in conclusive agreement with the earlier data from *Potentilla* of the rose family. The independent evidence from three species-complexes belonging to diverse plant families now establishes firmly the concept of genetic coherence as a principle operating in the evolution of higher plants.

The evidence from *Achillea* comes from a cross between contrasting latitudinal races of *A. borealis*, a dwarf form from Kiska Island at 52°N latitude, and a giant race from the San Joaquin Valley at 36°N. The F₂ progeny from this cross segregated widely, showing various recombinations of the parental characters (cf. *Year Book* 51, pp. 122-124 and pl. 1). The inheritance of 9 characters distinguishing the parents was studied in 300 F₂ individuals. Of the 36 combinations possible among pairs of these 9 characters, 7 combinations, or 17 per cent, had values of r ranging from zero to 0.11, indicating random distribution. The great majority, 29 combinations, or 83 per cent, had r values above 0.11, indicating a significant degree of correlation at the 1 per cent level or better, and 15 of the 29 combinations had r values ranging between 0.25 and 0.64. Despite

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![Diagram](image)

**Fig. 28.** Genetic coherence in *Mimulus* as expressed by the frequency of individuals within a segregating F₂ population sharing in the expression of pairs of characters. The degree of coherence is indicated by the values of the correlation coefficient r computed from observations on 300 plants. Some of the character differences between the parents are shown by the drawings.
the great range of parental recombinations observed in the F2 population, there is an unmistakable tendency for F2 progeny resembling either one parent or the other to occur with higher frequency than would be expected statistically on the basis of free random recombination.

The data from the *Mimulus cardinalis-lewisii* complex provide even more convincing evidence of the reality of genetic coherence. Many of the ecological races of *Mimulus* are distinguished by vegetative and floral characters that serve as excellent markers. A cross between the red-flowered, broad-leaved form of *M. cardinalis* from 45 meters elevation near the California coast and the lavender-flowered narrow-leaved alpine *M. lewisii* from 3300 meters in the Sierra Nevada yields highly segregating F2 populations that exhibit conspicuous recombinations of the parental characters.

The average expression of 13 dissimilar characters that distinguish the parental races was determined over a 5-year period at the three altitudinal transplant stations. The values of the correlation coefficient \( r \) for all possible combinations of paired characters were computed. Results from the Stanford data are shown in simplified form in figure 28. Of the 78 possible paired combinations of the 13 characters scored, 73 combinations, or 94 per cent, are significantly correlated at the 1 per cent level, and only 6 per cent have values of \( r \) indicating random distribution. The statistical evidence unquestionably supports the reality of genetic coherence in *Mimulus*.

Genetic coherence appears to be the mechanism by which ecological races, subspecies, and species have become differentiated through natural selection over periods of geologic time. Through coherence, combinations of characters that have attained equilibrium by natural selection tend to be preserved; at the same time the wide range of recombinations that occur after hybridization provide extensive possibilities for selection and the evolution of new coherence systems that are in equilibrium with new selective pressures.

**Transplant Responses of *Mimulus* in Relation to Genetic Coherence**

Malcolm A. Nobs, William M. Hiesey, and Harold W. Milner

The tendency for morphological characters of *Mimulus* to be genetically linked with capacity for survival at the Stanford and Timberline transplant stations was noted in *Year Book 60*, pages 381–384. More complete data now available are summarized in figure 29, which presents the most important features relating to the performance of a genetically segregating F2 population of 300 cloned plants originating from a cross between the form of *M. cardinalis* from Los Trancos near Stanford and an alpine form of *M. lewisii* from near our Timberline station.

In the figure the performance patterns of the F2 plants are divided into the nonsurvivors at the left and the survivors at the right. This classification is based on 5 years' accumulated data. The nonsurviving individuals were replanted several times at each station in order to distinguish as closely as possible between failure due to accidental causes and failure due to the inability of the plant to cope with the climate.

The entire F2 population was classified into three groups: those tending to resemble the *M. lewisii* parent (black bars), those tending to resemble the lowland *cardinalis* parent (white bars), and intermediate types tending to resemble the F1 (crosshatched bars). The grouping of the F2 plants into these three categories is based on index values assigned to each individual plant on the basis of seven contrasting, essentially nonmodifiable vegetative and floral characters distinguishing the original parents.

For each transplant station, data on the F2 plants are arranged into classes according to the mean total dry weights of the above-ground parts of the plants at the end of the growing season. These
weights are taken as a measure of over-all bulk growth, or vigor, at each station. The relative positions of the lowland *M. cardinalis* parent (*P*₁), the alpine *M. lewisii* parent (*P*₂), and their *F₁* progeny are indicated by the arrows at each station.

At Stanford, where the alpine *M. lewisii* parent (*P*₂) is a nonsurvivor and the coastal *M. cardinalis* parent (*P*₁) a vigorous survivor, the *F₁* displays intermediate vigor. Among the *F₂*’s the percentage of over-all survival is excellent except for a relatively small fraction that morphologically resembles the *M. lewisii* parent. Among the survivors the most
vigorous are those resembling either the *M. cardinalis* parent or the F₁-like recombinations. The weaker survivors at Stanford consist mostly of F₂ individuals resembling the *M. lewisii* parent or recombinations resembling the F₁. The fact that there are a few *lewisii*-like F₂'s that have a fairly high degree of vigor and also a few *cardinalis*-like plants with low vigor is also noteworthy. The marked enhancement in vigor of a few F₂ individuals at Stanford as compared with the coastal *M. cardinalis* parent is also of considerable interest, indicating the capacity of the nonsurviving alpine *lewisii* parent to contribute something to the F₂ progeny which in certain recombinations enhances its growth in the Stanford environment.

At Timberline the relative performance of the same cloned F₂ progeny is markedly different. The proportion of nonsurvivors is much higher than at Stanford, the unsuccessful F₂ plants consisting mostly of *cardinalis*-like and intermediate F₁-like individuals. The lowland *M. cardinalis* parent (P₁) is clearly a nonsurvivor, and even the alpine *M. lewisii* parent is a relatively weak survivor in the transplant garden whereas the F₁ hybrid is quite vigorous. Among the surviving F₂ progeny in this rigorous environment the most vigorous as well as the weaker classes include F₁-like recombinations and *lewisii*-like individuals. The weaker classes include the few surviving *cardinalis*-like recombinations.

The situation at Mather is anomalous. A high proportion of the F₂ progeny are nonsurvivors, like both the lowland *M. cardinalis* and the alpine *M. lewisii* parents. The F₁, on the other hand, is highly vigorous. Among the survivors, the most vigorous are F₁-like recombinations together with an occasional *cardinalis*-like or *lewisii*-like individual. The weak classes include individuals of all three recombination types without bias in their relative frequencies. Of particular interest at Mather are, first, the complementary effect of adding the heredities of the nonsurviving lowland and alpine parents to produce an F₁ vigorous in this environment, and, second, the fact that the selective bias is clearly in favor of a very small segment of F₁-like types which appear to have inherited a certain critical physiological balance needed for survival in that environment. Neither *cardinalis*-like nor *lewisii*-like F₂'s are favored in the Mather environment—another manifestation of genetic coherence.

At Stanford coherence is expressed in the tendency for *cardinalis*-like F₂ progeny to be more vigorous than the *lewisii*-like recombinations, whereas at Timberline *cardinalis*-like individuals are eliminated in higher frequency than the *lewisii*-like progeny. The same coherence in the same cloned F₂ plants is thus expressed in opposite directions in these contrasting environments.

Early observations this year on transplant responses at Stanford and Mather of new plantings made last year of F₁ hybrid combinations between a series of ecologic races of *M. cardinalis* and *M. lewisii* already show some conclusive results. Not only do different F₁ hybrid combinations between various races of either *M. cardinalis* or *M. lewisii* show significant differences in their winter survival and subsequent summer growth at Stanford and Mather but also F₁ hybrids between almost any race of *M. cardinalis* and any of *M. lewisii* show outstanding capacity to survive at both Stanford and Mather. Such complementary enhancement in the F₁ strongly confirms some of the results reviewed above. The differences in performance among the F₁ progenies of various crosses reveal in clearer perspective the relative physiological importance of interracial as compared with interspecific differentiation in the *Mimulus cardinalis-lewisii* complex.
**Physiology of Climatic Races**

*Harold W. Milner, William M. Hiesey, and Malcolm A. Nobs*

In preceding Year Books we have described the photosynthetic responses of climatic races of *Mimulus cardinalis*. We found that six races, native in six widely differing localities, had different responses of photosynthetic rate to temperature and light intensity, and that under conditions for maximum photosynthesis the rates decreased to different extent with time. Comparable data are needed for climatic races of *M. lewisii* to complete a survey of the *M. cardinalis-lewisii* species-complex. Until recently such data were entirely lacking because of the inability of *M. lewisii* to grow satisfactorily in either the garden or greenhouse at Stanford.

During the past year encouraging progress has been made toward obtaining satisfactory *M. lewisii* plants. Experiments described below with our controlled environment growth cabinets are pointing the way to selection of conditions suitable for the growth of *M. lewisii*. Measurements of photosynthesis have been made on some of the cabinet-grown plants.

The still incomplete data obtained for four clones of the Timberline race of *M. lewisii* indicate that it reaches maximum photosynthesis at a little higher temperature, it requires a higher light intensity to saturate photosynthesis, and its photosynthetic rate declines more during 12 hours than the rates for races of *M. cardinalis*.

The plants used for measurements of photosynthesis were seedlings. Current emphasis is being placed on vegetative propagation of the seedlings as clones in the cabinets. It now appears likely that by means of the cabinets we can establish and maintain clones of a number of climatic races of *M. lewisii* for measurements of their physiological responses.

The growth responses of *Mimulus* in controlled environments emphasize the high sensitivity of these plants to external variables. Characteristic differences between climatic races of *M. cardinalis* are evident and can now be studied in detail with the present facilities. That similar differences also occur within altitudinal and latitudinal races of *M. lewisii* is becoming apparent from experiments with seedling material. Previously the racial differences within *M. lewisii* were obscured by the inability of any of the races to grow sufficiently well under uncontrolled conditions at Stanford.

Controlled cabinet studies using vegetatively propagated clones of diverse altitudinal races of *M. cardinalis* are being continued along the lines described in *Year Book 61*, pages 317–320. The variables studied include temperature, light intensity, and carbon dioxide concentration. Experiments with clones are now being supplemented with studies on seedling populations of races of *M. cardinalis* and *M. lewisii*.

Two cabinets are being built for operation with natural light in addition to the four cabinets with artificial illumination. The new cabinets will extend the range of combinations possible in experiments in which light intensity and spectral distribution will be important variables.

**Studies on Tissue Cultures**

*Frank Nicholson, Kathe Picken, and William M. Hiesey*

The work begun last year on methods of growing tissues of selected clones of *Mimulus* has advanced to the point where comparative growth studies can be started. Beginning in March 1963, Mrs. Picken was able to devote full time to the testing of various modifications of media for growing different kinds of *Mimulus* tissues. Mr. Hart has completed controlled temperature cabinets for growing liquid cultures in roller drums.

Experiments in establishing plant tissues from stem internodes, roots, stem tips, callus tissues, and sterilized seeds have been tried with *M. cardinalis* and to
a lesser extent with *M. lewisii*. In this work the basic medium of Laetsch and Briggs mentioned in Year Book 61, pages 323-325, has chiefly been used. More recently another medium described by Murashige and Skoog (Physiol. Plantarum, 15, 473-497, 1962), having a considerably higher concentration of inorganic salts and vitamins instead of coconut milk, was tried; it appears to have some special advantage for culturing tissues of *M. lewisii* seedlings.

As was mentioned last year, tissues from a number of races of *M. cardinalis* were established quite readily from peeled stem internodes in the Laetsch and Briggs medium. Subsequent work with various concentrations and combinations of supplements has substantiated these results and has extended our range of experience in handling material of this species. One modification containing 10.0 mg of proteose peptone and 0.5 mg of naphthaleacetic acid per liter (medium G) produced the greatest abundance of roots; another containing 0.1 mg per liter of indoleacetic acid (medium U) produced steady moderate growth of callus in a high percentage of the cultures; still another modification with 0.6 mg of 2,4-dichlorophenoxyacetic acid per liter (medium T) produced bizarre, rapid growth in a few cultures. Five other combinations of supplements produced less significant results.

In *M. lewisii* the above technique of starting with peeled internodes was much less successful than in *M. cardinalis*. Recent work with seedlings of *M. lewisii* germinated on agar has, however, yielded encouraging results that indicate the feasibility of growing clones of both *M. cardinalis* and *M. lewisii* on a comparative basis.

Seeds of the Timberline *M. lewisii* race (7405) were sterilized in 1 per cent hypochlorite solution, rinsed in sterile distilled water, and germinated on agar medium at room temperature. From these stock cultures either entire seedlings or parts of seedlings were transferred to liquid Laetsch and Briggs medium in roller drums. The parts transferred included roots, roots and lower portions of the main stem, the entire main stem minus the basal portion, the central portion of the main stem only, and the stem apex with one or two pairs of leaves attached. Both the intact seedlings and the assorted parts with the exception of the roots grew in liquid culture at room temperature in diffuse light. After a month they developed characteristic differences. Seedlings with an intact apex continued to elongate and produced a few side shoots, whereas those with the apex removed developed a great abundance of side shoots so that the cultures became a mass of stems, leaves, and, frequently, adventitious roots. Pieces of such material consisting of a terminal bud and two or three pairs of leaves continued to grow with reasonable vigor when transferred to fresh Laetsch and Briggs medium. Corresponding pieces of the same seedlings grown in the Murashige and Skoog medium produced longer internodes and larger leaves than those grown in Laetsch and Briggs medium.

Seedling cultures of *M. lewisii* in the same medium generally grow faster in liquid than on agar. There are also characteristic differences in color and mode of growth in the two even though the added nutrients are the same. For our purposes, both the solid and the liquid media appear to be useful, the solid for maintaining stock cultures, the liquid for comparative growth studies.

A simple method for rapid feeding of liquid cultures by a Cornwall automatic pipetting syringe was developed. It greatly facilitates maintenance of the relatively large number of cultures needed for comparative growth studies.

We intend to embark on our main program after establishing a stock of healthy, consistent root, callus, and seedling cultures. The objective of the program is to supplement knowledge of growth responses of intact plants that have been grown in controlled growth cabinets. Parts of these same cloned plants will be grown in tissue culture; their responses
will be studied and compared with those of the whole plants. In this way many aspects of the physiological characteristics of the ecologic races can be examined in the light of different sets of relationships. With these findings, and quantitative measurements on photosynthesis, we hopefully anticipate a new comprehension of how and why contrasting ecologic races function as they do in their widely differing natural environments.

In summary, we are well on the way to developing methods and equipment for systematic studies on the comparative growth and development of tissues of the key climatic races of Mimusus. We hope to be able to integrate the results with the other three principal approaches of our over-all program as outlined in the introduction of this report.

Studies on the Distribution of Tree Species
Jens Clausen

During a visit to Brazil in the fall of 1953 (Year Book 53, pp. 151, 162-164) the low altitudinal limits of tree growth on the southeast Brazilian mountain massifs were observed. Through the following years this observation led to a search of the botanical literature for information about the location of tree lines in regions around the earth. The search disclosed that the limits of tree growth are determined not only by altitude and latitude and other factors of the environment but to an even greater extent by the nature of the germ plasms of the tree species themselves, which differ greatly in their ranges of tolerance. Each tree species, accordingly, has its own tree line. Furthermore, the general tolerance range is a characteristic not only of the species but also of the genus, family, and taxonomic order.

The most widely distributed tree species belong to species-complexes and genera that circle the earth within characteristic latitudinal limits. Each species-complex or genus has a common gene pool. The most hardy trees of high latitudes are conifers of the pine family, aspens, willows, birches, alders, and the mountain ashes. They compose a small elite of 12 species-complexes that are limited to the northern hemisphere. Cedars, oaks, chestnuts, beeches, and eucalypts belong to a group of a few thousand species of medium tolerance that compose the forests at medium latitudes of the northern and southern hemispheres.

By far the largest number of tree species on the earth, possibly 50,000 or more, compose the forests within the low latitudes. These species are limited roughly between 25°N and 25°S. They are members of families and orders that are extremely sensitive to frost and through the evolutionary periods have remained within tropical climates.

A fair number of tree genera are intermediate between the three major groups. Also, within each complex of species the genetic variability and recombination enable individual species to adjust to ecologically diverse situations within the latitudinal belt. Most orders and families of trees, however, have not escaped the zones to which their germ plasms generally are adjusted. This observation suggests the existence of evolutionary limitations within many families and orders.

The families and orders are based on morphological criteria. The strong regional limitation of orders and families suggests that deep-seated physiologic-hereditary differences are associated with the morphological ones.

Population studies of trees in the Harvey Monroe Hall Natural Area. During the late summer months of 1960-1962, population studies were conducted on tree species near the tree line in the Slate Creek Valley of the Harvey Monroe Hall Natural Area surrounding the Timberline transplant station. Approximately 150 population samples containing more than 36,000 individual trees were classified as
to species and variation in growth form within species in relation to altitude and slope exposure.

The Harvey Monroe Hall Natural Area is within the Inyo National Forest and adjoins the east side of the Yosemite National Park. It is composed of approximately 7 square miles of rugged terrain at altitudes of 3050 to 3900 meters on the east slopes of White Mountain, Mount Conness, and North Peak (cf. Year Book 32, pp. 20–21, 180). Three glaciated valleys run west to east, providing north- and south-facing slopes which have contrasting climates. A varied topography, including rock slopes, talus, seres, alpine pavements, glaciers, cirques, small lakes, streams, waterfalls, bogs, meadows, gravel beds, and a small lava cone, provides a highly diversified array of habitats for plant and animal life. The area has an unusually rich vegetation—about 350 species of flowering plants and ferns—for such a high altitude.

Three conifers, whitebark pine (Pinus albicaulis Engelm.), lodgepole pine (P. murrayana Grev. et Balf.), and mountain hemlock (Tsuga mertensiana (Bong.) Kerr.), have tree lines within the Hall Area. Each develops large trees up to near the tree line, which are followed by knee-high cushions of elfinwood higher up. In the tree-line zone the growth forms of each of the three species are highly intermixed. The existence of contrasting forms in juxtaposition suggests that the differences are primarily genotypic. The tree line of a species may be defined as the altitude beyond which it no longer exists as a tree. The species may exist at higher elevations in the form of a low cushion, or even as a carpet.

Slate Creek Valley within the Harvey Monroe Hall Natural Area is well adapted for the study of the dynamics of tree lines. As the glaciers of the Pleistocene period retreated toward the cirque in the westernmost part of the valley, they left approximately 11 terminal moraines, which traverse the valley in a south-north direction. The trees follow the moraines across the valley, from the tree line on the north-facing slope to the tree line on

Fig. 30. Examples of growth habits of Pinus albicaulis near tree line in the Harvey Monroe Hall Natural Area. 1. Elfinwood. 2. Intermediate form with elfinwood base and dead erect trunks. 3. Erect multitrunk tree (most common form). 4. Single-trunk tree (rare). The scale is in meters. Cf. tables 5 and 6.
the opposite south-facing slope. Abrupt rock escarpments limit individual populations on the moraine.

The 150 population samples taken varied in area between 20,000 and 90,000 square meters, and included from 100 to 1800 trees per sample. In each sampled area all trees, including small seedlings, were counted. Trees having trunks less than 25 cm in diameter seldom have cones and were classed as immature. Among the mature trees a giant class was recognized having trunks more than 75 cm in diameter. All three species have trees up to 150 cm in trunk diameter. The largest tree, a hemlock, had a trunk diameter of 2 meters.

The mature trees were subdivided into groups on the basis of growth form as shown in figure 30. Each species has both single-trunk (fig. 30, 4) and multitrunk growth forms (fig. 30, 3), differing in frequency among the three species. The occurrence of elfinwood (fig. 30, 1) and of intermediates of many combinations indicates that tree line is approaching. The elfinwoods appear to be genetically distinct forms, although direct experimental evidence for this deduction is lacking. The most interesting intermediates are forms having elfinwood at the base combined with erect trunks (fig. 30, 2). In the vicinity of tree line the erect trunks are often killed, but the elfinwood bases survive and continue to bear cones, evidence of the severe environment for tree growth and also of the survival value of the elfinwood growth form.

Six samples listed in table 5 illustrate the trends found in these population studies, three series from north-facing

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<tr>
<th>Growth Form*</th>
<th>3065- to 3080-m alt.</th>
<th>3125- to 3140-m alt.</th>
<th>3265-m alt.</th>
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<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
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<tr>
<td>1. Elf in wood</td>
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<td>44.3</td>
<td>73.4</td>
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<td>2. Intermediate</td>
<td>---</td>
<td>25.5</td>
<td>12.5</td>
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<td>3. Multitrunk</td>
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<td>16.8</td>
<td>---</td>
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<td>4. Single trunk</td>
<td>4.6</td>
<td>2.8</td>
<td>---</td>
</tr>
<tr>
<td>5. Immature</td>
<td>40.5</td>
<td>23.6</td>
<td>10.7</td>
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<tr>
<td>Total %</td>
<td>73.6</td>
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<td>97.3</td>
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<th>3140- to 3155-m alt</th>
<th>3290-3360-m alt</th>
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</tr>
<tr>
<td>1. Elf in wood</td>
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<td>29.2</td>
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<td>2. Intermediate</td>
<td>---</td>
<td>---</td>
<td>60.1</td>
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<td>3. Multitrunk</td>
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<td>4. Single trunk</td>
<td>8.1</td>
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<td>5. Immature</td>
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<td>83</td>
<td>882</td>
<td>111</td>
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Table 5. Composition of Samples of Populations of Pinus Species, Slate Creek Valley

* See figure 30.
slopes and three from south-facing, both series progressing in altitude. The floor of the Slate Creek Valley at 3050 meters is used as a reference point.

*Pinus albicaulis* dominates on the north-facing slope (table 5), although large trees of *P. murrayana* also occur at altitudes up to 3080 meters. Below this altitude all trees are erect. At the 3125- to 3180-meter altitude only rare immature trees of *P. murrayana* with dead tops remain. Here many elfinwood and intermediate forms of *P. albicaulis* make their appearance in juxtaposition to erect, multitrunk trees 10 meters tall. Extreme elfinwoods are less than a meter high but up to 20 meters wide, as suggested in figure 30, 1. Many forms of vigorous intermediates exist here with or without elfinwood base; some intermediates have as many as 30 slender, erect trunks on a single bush. At 3265 meters altitude on the north-facing slope only elfinwood forms and dwarfish intermediates with elfinwood base are found, and at higher elevations only elfinwoods.

The situation is different on the south-facing slope (table 5, B, 1). In two samples from altitudes at 3065-3110 and 3140-3155 meters only 5 to 8 per cent of the trees are *P. albicaulis*, but here *P. murrayana* is exceedingly vigorous and develops large trees up to the 3200-meter level. At 3290-3360 meters *P. albicaulis* is dominant, consisting predominantly of elfinwood and intermediates having elfinwood bases. *P. murrayana* at this elevation has also evolved typical elfinwood plants.

The east-facing slopes (table 6) are similar to the south-facing slopes in the ratio of the frequency of *Pinus albicaulis* to *P. murrayana*, but the hemlock, *Tsuga mertensiana*, contributes a moderately conspicuous element. The growth forms of the hemlocks are as varied as those of the whitebark pine, ranging from single- and multitrunk trees through intermediates to low elfinwood types that are draped over the rocks.

A total of 11,626 trees of the *Pinus albicaulis*, 24,201 *P. murrayana*, and 644 *Tsuga mertensiana* were found among 150 population samples enumerated. In addition, *P. jeffreyi* Grev. et Balf. was represented by 5 small and immature although probably very old individuals, *Juniperus communis* L. by numerous specimens all of which were elfinwood, and the chinquapin, *Castanopsis sempervirens* (Kell.) Dudl., by a single elfinwood plant.

Each of the three most frequent species of conifers in the Harvey Monroe Hall Natural Area has its specific tree line, and each differs also in its slope preference. The hemlock, *Tsuga*, is limited primarily to steep, east-facing slopes between 3000 and 3125 meters in altitude, but single individuals were found in most of the samples, and cone-bearing elfinwood specimens occur up to 3375 meters. The lodgepole pine, *Pinus murrayana*, predominates on warm east- and south-facing
slopes, where its tree forms extend to 3200 meters. Its elfinwood form is rare and limited to certain pockets. The white-bark pine, *P. albicaulis*, has its tree limit at 3200 meters on north-facing slopes and 3300 meters on south-facing slopes, but the elfinwood forms ascend to the 3600- to 3700-meter levels.

All three species have an altitudinal transitional zone of approximately 60 meters within which multitudes of recombination types between the erect and elfinwood races intermingle. The situation appears to be a highly dynamic one, in which the lower- and higher-altitude races appear to interbreed and segregate, a tension zone where severe natural selection operates. The existence of intermediate steps between the extremes suggests an expression of the interaction between many genes and environmental modification in the intermediates.

The willows, members of the genus *Salix*, have counterparts of the variants observed among the conifers. *Salix eastwoodiae* Ckll. exists only as a tall bush up to approximately 3400 meters altitude. In contrast, *S. petrophila* Rydb. and *S. nivalis* Hook. are “lawn” types that creep among the grasses and drape themselves over rocks up to high altitudes. Two species, *S. monica* Bebb. and *S. orestera* C. K. Schneid., behave like the conifers. At 3050 meters these species are erect bush types 1 meter tall, but at 3080 meters populations of these species are composed of aggregates of matted lawn types interspersed by erect to divaricate bush types. Classification of a sample of 157 mature plants within an area 300 meters long beside Slate Creek indicated 77 plants of the horizontal lawn type as compared with 80 erect to intermediate plants. Some of the erects were found growing in the middle of lawn-type plants, suggesting genetic distinctness.

Within the Harvey Monroe Hall Natural Area, accordingly, five species have evolved parallel, highly variable populations near the limits for their erect tree and bush forms. Such transitional forms precede the actual tree line, which is at a slightly higher altitude. The variability in the intermediate populations is high.

**Cytotaxonomy and Distributional Ecology of Western North American Violets**

*Jens Clausen*

During the years from 1932 to 1943 (*Year Book 36*, pp. 213–214) the chromosome numbers of most of the western North American violets were determined. This investigation was conducted in cooperation with the late Professor Milo S. Baker. Since the results had not previously been organized and published, the task was undertaken this year.

Of the 14 taxonomic sections in the genus *Viola*, 4 occur in western North America. The section *Chamaemelanium* has its center of variation in this area. It is a relatively primitive yellow-flowered group having a basic chromosome number of 6. All levels of polyploidy are represented, from the diploid \( n = 6 \) to the duodecaploid \( n = 36 \), and at least 7 evolutionally distinct polyploid lines have been established within the section.

Another section is *Plagiostigma*, a group of meadow violets having the chromosome numbers \( n = 12, 24, \) and 48 in multiples of 12. The eastern stemless blue violets constitute a separate group within the section. They uniformly have \( n = 27 \) pairs of chromosomes. The stemmed blue violets of the *Rosatellata* section follow a 10 series, having \( n = 10, 20, \) and 40 chromosomes. The only North American member of the *Melanium* section, the pansies, has \( n = 17 \) pairs of chromosomes.

The existence of ecologically distinct subspecies that are adapted to different habitats across the west has enabled the violets to occupy nearly a full range of environments within western North America without change in chromosome number. Superimposed on this pattern of diploid distribution are series of polyploid
species and likewise series of sections having different basic numbers. All these species are able to coexist without interbreeding. Distinct sections, accordingly, have been able to superimpose their polyploids on the diploids of simpler differentiation. The *Viola* genus is one of the most versatile groups of higher plants.

**SPEECHES**


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