Proc. Nat. Acad. Sci. USA
Vol. 68, No. 11, pp. 2893–2897, November 1971

The Distribution and Action in Photosynthesis of Several Forms of Chlorophyll

C. S. FRENCH

Carnegie Institution, Stanford, California 94305

This symposium commemorates the discovery by Priestley 200 years ago that plants somehow reconstitute air that has been used by animals. Priestley's finding preceded the discovery of oxygen and carbon dioxide, so there was no adequate chemistry known then to help interpret this striking observation. This effect of plants on air was the beginning of an understanding of the relationships between different kinds of living things. For this reason, Priestley should be considered an early contributor to the popular field of ecology, as well as the discoveror of the process we now call photosynthesis.

200 years seems like a very long time when we consider the rate at which science is expanding. I note, however, that all the speakers on this program have been concerned with photosynthesis problems for somewhere between a tenth and a fifth of the time that has elapsed since Priestley's initial discoveries. This occasion is an appropriate time to comment on some of the changes that have taken place in the life of scientific investigators and the way these changes of environment bear on the subject of photosynthesis. The time scale of scientific progress has been so nonlinear that most of the differences in the way scientific research is done now and in Priestley's time have taken place during our lifetimes. The recent increase in the sheer volume of relevant subject matter has been overwhelming. For instance, in the late 1930's and early 1940's it was possible for one person to read, understand, and review critically most of the world's literature on photosynthesis. In fact, Professor Rabinowitch did so with remarkable success. By now the different aspects of photosynthesis: the physics, the photochemistry, the biochemistry, the plant physiology, and the ecology have each grown beyond the capacity of single individuals to thoroughly comprehend. The change in the quantity of information has been great, but an effect of even greater magnitude on the environment of the research worker has been the increased support for basic studies. Science has become a way of life for large numbers of people.

In the 1930 decade, about six million dollars was committed to build the Palomar telescope. I presume that this sum was by far the largest amount of money that had ever been devoted to any one piece of scientific research equipment up to that time. It then seemed like a perfectly fantastic concentration of funds on one aspect of science. At about this time, Jack Myers and I discussed an equally fantastic notion—the effects that a million dollars for research might have on the field of photosynthesis. There must have been about a dozen or perhaps 20 people in the world then engaged in photosynthetic research. The thought then was that an effort on a million dollar scale would solve all the problems that one could reasonably ask about the subject. Since then, of course, that much has been spent in a good many different places to find out about the mechanism of photosynthesis; the yield of information and understanding has been high. As a result, the number of questions remaining to be solved has increased rather than decreased because far more significant questions can now be asked.

In Priestley's time, the question was "What is this process by which plants make old used up air again useful to animals?" Now, however, the question we are asking is "What is the molecular mechanism by which the process of photosynthesis operates?" We are, therefore, concerned with the chemical nature of the substances participating in the process. Because of its widespread occurrence and striking color, the substance most spectacularly concerned with photosynthesis is chlorophyll.

The chemistry of chlorophyll extracted from leaves with alcohol or other organic solvents is well known. However, this extracted chlorophyll is only part of the complex existing in living cells. In its functional state, chlorophyll is combined with proteins as insoluble particles that contain also lipids, carotenoids, and even carbohydrates. The main reason for our ignorance about the chemistry of this natural green coloring matter which absorbs the sunlight used to drive photosynthesis is that the material itself is not soluble, and hence cannot easily be prepared in pure form for chemical analysis.

That there are several different forms of natural chlorophyll complexes is very clearly evident from the absorption spectra of green material in any water extracts of leaves or algae. The number and identity of these different forms is, however, not easy to deduce from inspection of curves without the curve analyses that will be the main topic of this discussion. The general idea that there must be a variety of different forms of chlorophyll in plants goes back at least to the Russian plant physiologist, Lubimenko, who expressed this idea in 1927. A number of investigators in many countries have been deeply concerned with the problem.

The leaves of most land plants are very much alike in their content of the different forms of chlorophyll. We have, therefore, found it far more profitable to investigate the spectra of chlorophyll from microscopic algae, because their range of variation is much greater than that of leaves.

Whole algae or intact chloroplasts are so optically dense that the spectra of their suspensions are distorted. The light leaking around the particles gives an apparent absorbance that becomes more erroneously small the higher the actual absorbance. In order to get around this peak-flattening distortion and also to make it possible to separate from each other the particles comprising the different photochemical systems, we use only very small particles of disintegrated chloroplasts. The disintegration can be done by extruding the suspension of live algae through a needle valve. Other methods of disintegration that have been successful are detergents, supersonic treatment, shaking with glass beads, or grinding with fine powdered abrasives. After disintegration, the material is centrifuged to

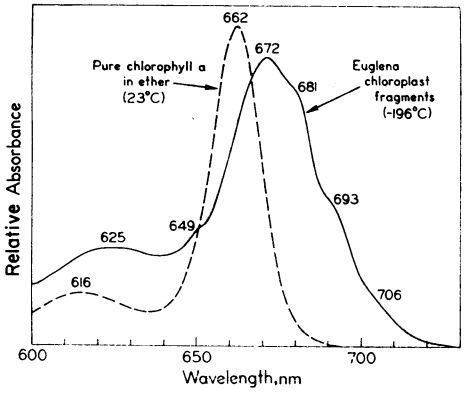

FIG. 1. The spectrum of the alga *Euglena* shows bands due to four different forms of chlorophyll *a* at 672, 681, 693, and 706 nm. Each of these forms has its own characteristic spectrum, presumably somewhat similar to that for isolated chlorophyll *a* (*broken line*), but shifted in wavelength. The small band at 649 nm is due to chlorophyll *b*.

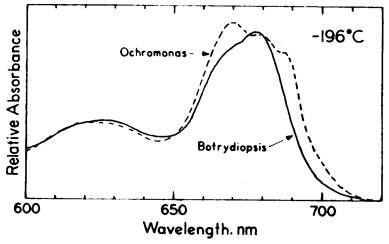

FIG. 2. Absorption spectra of two algae lacking chlorophyll *b* that suggest the same chlorophyll *a* forms occur in different species, but in varied proportions (Courtesy of Dr. J. S. Brown).

get rid of the cell walls and unbroken chloroplasts. Even so, the resulting material does scatter some light, so conventional spectrophotometers give somewhat distorted results. A thin cell using the opal glass window principle of Shibata, with a metal spacer, when placed in front of a large aperture photomultiplier, gives reasonably good spectra. This cell holder may be used at low temperature by dipping the lower part of the metal spacer into liquid nitrogen. An absorption spectrum (1) is shown in Fig. 1 for a preparation from *Euglena*, where it is compared with the absorption spectrum of chlorophyll dissolved in diethyl ether. Here we see that the native chlorophyll in its functional state has a complex spectrum consisting of a number of overlapping absorption peaks. Our problem is to

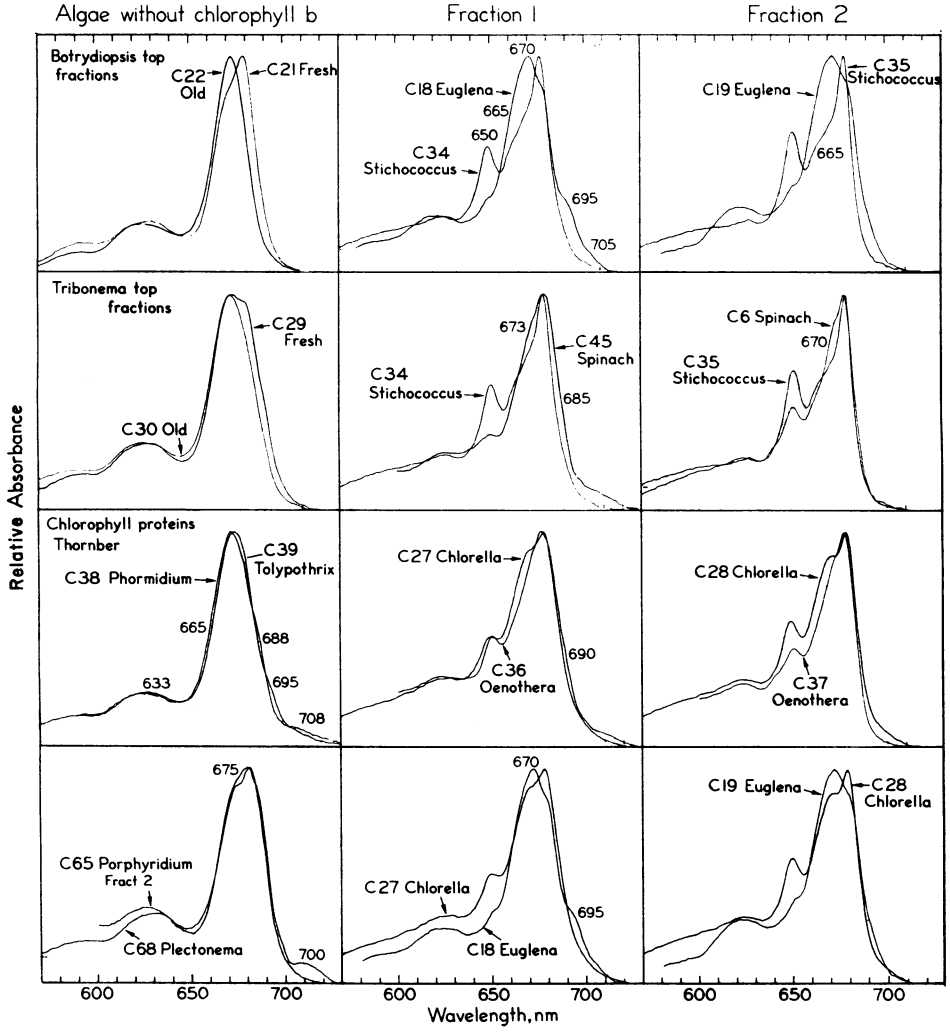

FIG. 3. Some selected spectra of algal homogenates or chloroplast fractions showing that there are various forms of chlorophyll *a*. (2)

resolve complex curves like this into their basic components. To do so requires the comparison of spectra from many different algae.

Fig. 2 shows two liquid-nitrogen absorption spectra of algae containing only chlorophyll *a*. Here we can compare *Ochromonas* and *Botrydiopsis*. It looks as though the *Ochromonas* spectrum was made of two components that were present also in *Botrydiopsis*, in addition to another long-wavelength one, which may or may not be present in small amounts in the *Botrydiopsis* spectrum. Fig. 3 shows comparisons of fractions 1 and 2 from several algae and leaves. The sharp peak at 650 nm is due to chlorophyll *b;* a 640-nm form of chlorophyll *b* is also evident in some species.

These curves illustrate some of the complexities that pose the question: "Can this variety of spectra be obtained from the summation of a comparatively small number of invariant, universal components?" Several concepts have been proposed to be more specific about the various ideas that have been used to describe the shape of the chlorophyll spectra in its native state (2). The "constant component concept" maintains that the components themselves have peaks of constant wavelength and the observed variety of spectra is due to different proportions of a small number of definite forms. The "extra components concept" states that the major peaks may be due to components of constant wavelength, but there may be also a variety of other extra components. The third and more complex idea is that the components may have peak wavelengths and widths that vary from one sample to another, although probably within a given range for each type of component. This concept boils down to the general idea that the spectra cannot be resolved into a small number of definite components. The results here summarized* support the constant components idea for constancy of wavelength, but the widths are not constant. Furthermore, "extra" components are needed only at long-wavelengths. Thus, we do not need to assume an assortment of extra components in the part of the spectrum occupied by the four "universal" forms which will be discussed.

One of the major objectives of many different groups now is to separate the particles responsible for the two different photochemical systems involved in photosynthesis. A method developed by Michel and Michel-Wolwertz (3) for partially separating these two photochemical systems depends on disintegration of the material by extrusion through the needle valve followed by centrifugation on a sucrose gradient. The results of such a centrifugation give a green band near the top, termed fraction 1, which contains the system 1 particles and a lower one, called fraction 2, that contains primarily the larger system 2 particles, but still has some system 1 activity. The region around 680 nm is much more strongly absorbed by the fraction 1 than by the fraction 2 particles and the height of the chlorophyll *b* peak is much greater in the fraction 2 particles. We would like to know how many different pigments are involved in each of these complex particles and what the absorption spectra of each of the individual components might be. What used to be considered the broad and variable *Ca* 670-nm band (4) now is believed to be composed of two narrower components that occur in variable proportions.

Rather than using the older idea of two major components *Ca* 670 and *Ca* 680 nm, we now have much more success in

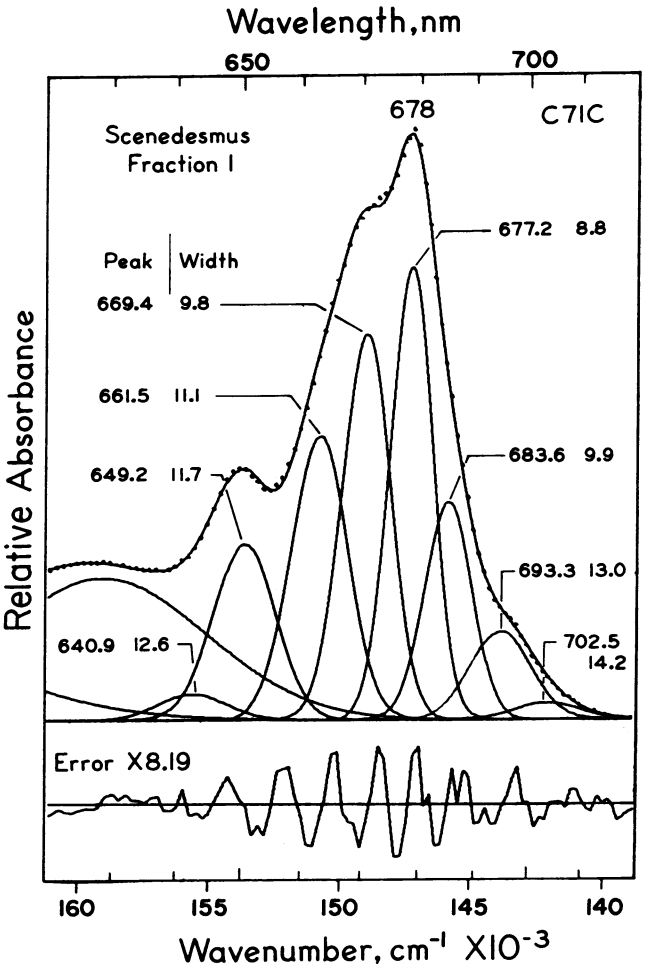

FIG. 4. The absorption spectrum at −196°C of fraction 1 from *Scenedesmus* chloroplasts fitted by the sums of Gaussian components. The observed data are plotted as points, while the line through them is the sum of the component curves whose characteristics are given in Tables 1 and 2. The error of fit at each point is shown below on a scale with the designated magnification.*

fitting these curves with four major chlorophyll *a* components, in addition to the long-wavelength pigments* (5, 6). Fig. 4 shows a curve analysis with a chlorophyll *b* peak at 650 nm and a much smaller chlorophyll *b* form at 640 nm. The major chlorophyll *a* components are at 662, 670, 677, and 683 nm. There are also two longer wavelength chlorophyll forms, at 692 and at about 705 nm. The last one is usually very small. We tried to match the spectra of many different preparations using these four "universal" major chlorophyll *a* components. The results have shown quite clearly that these four major components, with long-wavelength pigments near 692 and 705 nm when necessary, will give good fits to many spectra. Table 1 shows the range of wavelengths found for each of these forms with the standard deviations that resulted from fitting 15 different spectra, some of which are illustrated in Fig. 5. The peak wavelengths agree remarkably well with each other and lead us to believe that these numbers really define four specific major chlorophyll components.

The widths of these forms are, however, not constant as shown in Table 2. The components giving good fits to the *Stichococcus* preparations, and to several other algal spectra

* French, C. S., J. S. Brown, and M. C. Lawrence, submitted to *Plant Physiol.*

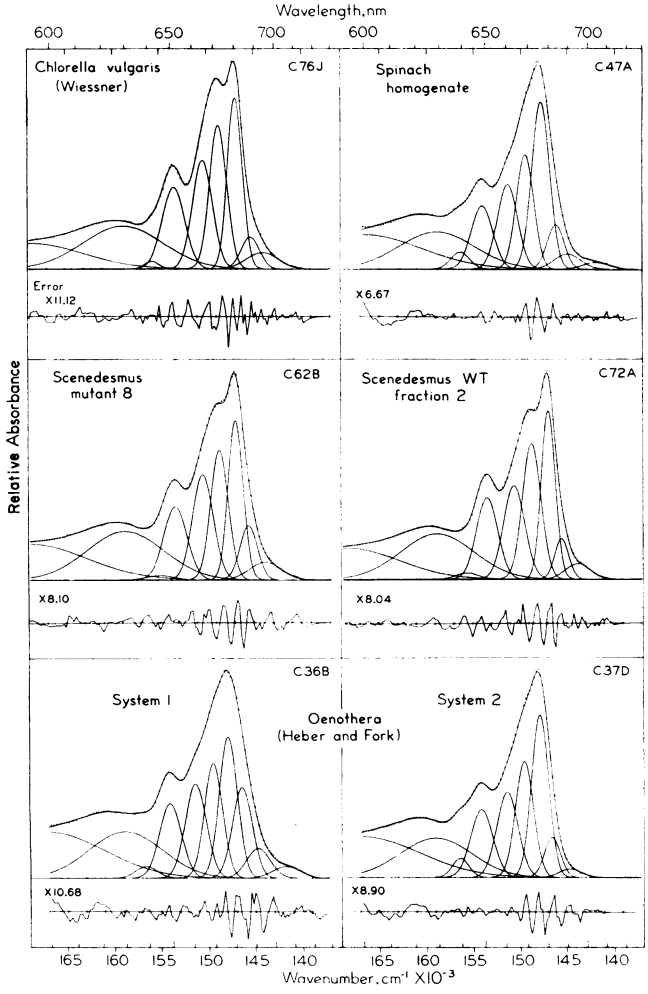

FIG. 5. Curve analyses of fractions of algal chloroplasts with absorption spectra typical of green algae and leaves. These, and similar or sharper, spectra were used to derive the component bands summarized in Tables 1, 2, and 3.*

that are very sharp, differ significantly in width from those for typical green algae and leaves. Furthermore, the widths of the 678- and of the 683-nm components vary between fraction 1 and fraction 2 preparations. The relative proportions of these different forms of chlorophyll *a* are shown in Table 3 as a percentage of the total absorption due to chlorophyll *a*. In fraction 1 the proportions of the 662- and the 670-nm forms are about equal and they are also about the same in fraction 2 as in fraction 1. However, there is about twice as much of the

TABLE 1. *Peak wavelengths of chlorophyll a forms (nm)*

Form of chlorophyll (nm)	662	670	677	683	692	705
Number of spectra	15	15	15	15	15	5
Maximum	662.3	669.8	677.8	685.1	693.7	704.4
Minimum	661.3	669.2	676.7	683.0	687.6	701.0
Standard deviation (±)	0.09	0.05	0.09	0.16	0.39	0.90
Average	661.6	669.6	677.1	683.7	691.5	704.6

TABLE 2. *The half-widths of different forms of chlorophyll a (nm)*

Form of chlorophyll (nm)	662	670	677	683	692	705
Unusually sharp algal spectra						
Fraction 1	11.0	9.3	8.6	10.6	17.0	14.2
Fraction 2	11.1	9.7	7.8	8.5	17.1	—
Typical green algae and leaves						
Fraction 1	11.3	10.0	10.3	10.8	13.0	18.7
Fraction 2	11.6	9.8	9.4	9.6	16.1	—

683-nm component in fraction 1 than in fraction 2, and the longer wavelength components are also more abundant in fraction 1. The main difference, however, is in the 683-nm component. Thus, with these four major universal forms of chlorophyll *a*, with two forms of chlorophyll *b* when necessary, and a few extra components at long wavelengths, we are able to satisfactorily interpret many different plant spectra. So far we have seen no evidence for any extra forms of chlorophyll in the spectral region between 640 and 690 nm.

It will be of great interest to compare the action spectra for the two photosystems with the room-temperature absorption spectra when both these types of data have been resolved into their component consituents by curve analysis.

The component bands so far discussed have been derived from measurements at liquid-nitrogen temperature on fractions of algal chloroplasts.

An attempt (7) to use the bands derived from sharp spectra to fit diffuse spectra was made by the comparison of fraction 1 and fraction 2 preparations from *Stichococcus* that had been measured both at liquid-nitrogen and at room temperature. The bands derived from analysis of the low-temperature spectra were used as input bands for the room-temperature spectra of the same samples. The resulting curve analyses of Fig. 6 showed an obvious broadening of the bands at room temperature. The two broad bands of the shorter wavelengths show no significant difference in width at the two temperatures. The increase in width at room temperature is about 3 nm for the main bands, other than that at 683 nm. The relative heights of the 650-, 662-, 670-, and 677-nm bands are in about the same proportion in the spectra at the two temperatures as may be seen by visual inspection of the curves. If these components derived by curve analysis represent actually existing forms of chlorophyll, they should be in the same proportion at the two temperatures. However, the somewhat hypothetical relative proportions of the components as calculated from their areas shows about as much correlation between the two fractions at one temperature as it does between the same fractions at the two temperatures. There is a lack of agreement in the height of the 683-nm band, which is greater in the room-temperature spectra than in the low-temperature spec-

TABLE 3. *Approximate distribution of the forms of chlorophyll a in two fractions (%)*

Form of chlorophyll (nm)	662	670	677	683	692	705
Fraction 1	22	23	29	17	7	3
Fraction 2	25	27	33	8	5	0

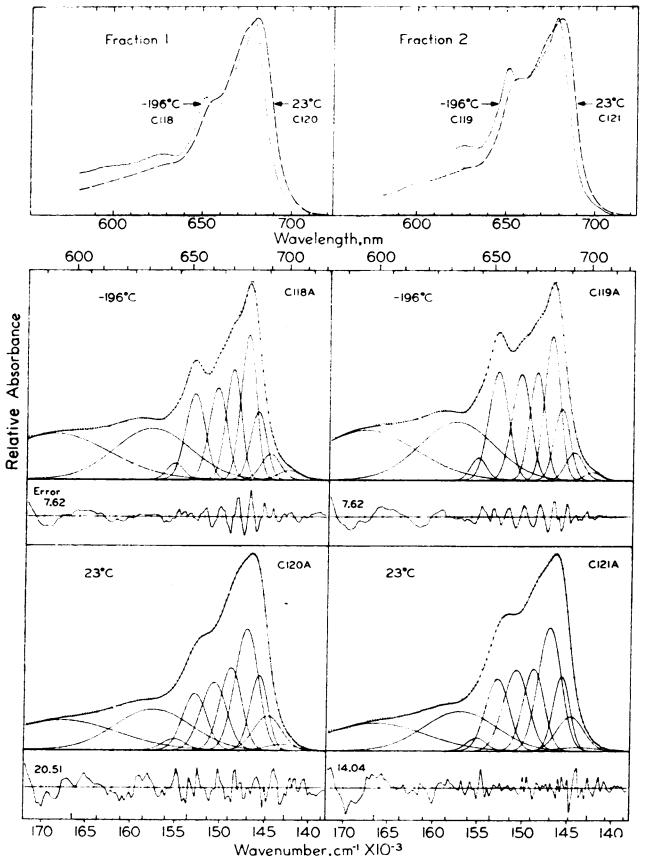

FIG. 6. Absorption spectra of *Stichococcus* fractions at two temperatures and the component bands that add to fit the measurements (7).

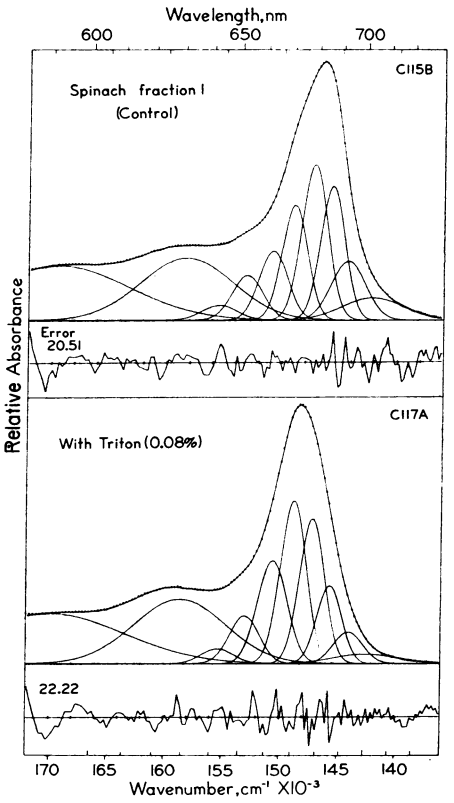

FIG. 7. Absorption spectra and their component bands of fraction 1 from spinach chloroplasts with and without Triton at room temperature. Triton converts the material absorbing at longer wavelengths to material with the same peak absorption wavelengths and widths as the previously existing short-wavelength forms of chlorophyll. The long-wavelength forms only possess the system-1 activity.

tra. This apparent discrepancy in height may possibly be due to the fact that this band was not widened as much at room temperature as were the others.

In summary, we may say that the relative proportions of the main bands, excluding *Ca* 683, are more or less reasonably approximated at the two temperatures, but that a precise measure of the relative proportions of the different forms in room-temperature spectra cannot yet be made by curve analyses of low-temperature spectra.

Triton is well known to increase the activity of chloroplast fractions for cytochrome *c* oxidation. This treatment also changes the spectrum strikingly. The apparent difference is a reduction of the long wavelength bands accompanied by an increase in the height of the shorter wavelength bands. To make a quantitative study of this situation, Dr. Brown and I have analyzed the room temperature spectra of spinach fraction 1 with and without .08% Triton (7). These curve analyses are shown in Fig. 7. The proportions of the 662- and 670-nm forms of chlorophyll have been increased by the Triton treatment, whereas the 683-, 692-, and 700-nm forms have been greatly decreased. The important thing that has come out of this comparison is that the new material generated by Triton from the long wavelength bands is not some unusual form of chlorophyll, but it has the same peak wavelength and halfwidth as the previously existing 662- and 670-nm forms of chlorophyll found in the untreated preparations. This suggests that the long wavelength bands are aggregates of shorter wavelength components that are disrupted by the detergent treatment.

The major challenge in studies of chlorophyll forms by curve analysis at the present time is to bridge the gap between the definite and reproducible results from data at liquid-nitrogen temperature, where the spectra are sharp, and the more diffuse room-temperature spectra. This is a particularly important question because the analyses of room-temperature spectra must be used as a basis for interpreting action spectra for the two photochemical systems.

CIW-DPB Publication No. 485.

1. French, C. S., M.-R. Michel-Wolwertz, J.-M. Michel, J. S. Brown, and L. K. Prager, *Biochem. Soc. Symp.*, **28**, 147 (1969).
2. French, C. S., *Carnegie Inst. Washington Year b.*, **68**, 578 (1970).
3. Michel, J.-M., and Marie-Rose Michel-Wolwertz, *Photosynthetica*, **4**, 146 (1970).
4. French, C. S., and Lillian Prager, *Progr. Photosynthesis Res.*, **2**, 555 (1969).
5. French, C. S., J. S. Brown, W. Wiessner, and M. C. Lawrence, *Carnegie Inst. Washington Year b.*, **69**, 662 (1971).
6. Wiessner, W., and C. S. French, *Planta*, **94**, 78 (1970).
7. French, C. S., J. S. Brown, and M. C. Lawrence, *Carnegie Inst. Washington Year b.*, **70**, in Press (1971).