INVESTIGATION OF THE FLUORESCENCE SPECTRA OF CHLOROPHYLLS A AND B IN ETHER SOLUTION

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With 2 Text-figures

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Many physiological researches have dealt with the rate of photosynthesis in the green plant cell under various conditions, and with the gross characteristics of the BLACKMAN "dark reaction" and the light reaction, which are the steps composing the photosynthetic process. The chemical researches on the plant pigments have been confined chiefly to the study of their molecular composition and structure, while very little experimental consideration has been given to these important compounds from the photochemical point of view. The detailed kinetics of the energy transformation from radiation to the carbon dioxide-water complex, the kinetics of the action of chlorophyll during carbon fixation in the green leaf, and the specific rôles of the different chlorophyll components in photosynthesis are completely unknown. Nor is it known whether the yellow pigments, the isomers of carotene and xanthophyll, are photosynthetically active.

Chlorophyll solutions at room temperature absorb radiant energy of all wave-lengths in the visible spectrum; the fraction of the incident energy absorbed however, is different with different wave lengths. This energy absorbed by the chlorophyll molecule may take several courses: — (I) its absorption may raise the chlorophyll molecule to such an energy level that it will react with a molecule of carbon dioxide, water, carbonic acid, or other nearby molecule; the complex may later dissociate into unactivated chlorophyll and an energy-rich compound, (2) the absorbed energy held by the activated chlorophyll molecule may be transferred as potential energy to other molecules by inelastic collisions, or so-called collisions of the second kind, (3) it may be dissipated directly as heat by inelastic collisions with surrounding molecules, collisions in which the potential energy of activation is changed to kinetic energy, or (4) the absorbed energy may be emitted by the chlorophyll molecule as fluorescent radiation. The chlorophyll molecule, after being previously raised to a higher energy state by absorption of light, probably loses energy by all of these paths in the green plant cell. In the first two processes, the potential energy of the chlorophyll, obtained by light absorption, is eventually transferred to other molecules. The reactions

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occurring during the processes of photosynthesis in which chlorophyll has a photochemical rôle undoubtedly consist of one or both of processes (1) and (2) listed above; the energy-rich compound of (1) would be the first carbon compound of photosynthesis, and in (2) the molecules which gain energy by transfer from activated chlorophyll would be those of a carbon dioxide-water complex.

Fluorescent radiation is emitted when an activated molecule, i. e., one in a higher energy state, assumes a lower energy state (electronic, vibrational, or rotational) without transfer of this energy to another molecule by collision. Studies of simpler systems show that the fluorescence intensity of a substance in solution may be decreased by collisions of the second kind with other suitable molecules. The fluorescence is then said to be damped. When photosynthesis is not in progress, it is therefore to be expected that a greater amount of the absorbed energy will be lost by fluorescence. The energy changes possible within the chlorophyll molecule, of which the fluorescence is visible evidence, would be more clearly understood if accurate quantitative data on the fluorescence and absorption spectra of chlorophyll were available. Such data, obtained from a study of artificial solutions and the green plant cell, would very probably provide insight into the photosynthetic mechanism. In earlier work reported in another paper (7), the absorption spectra of chlorophylls *a* and *b* in ether solution were studied by use of an accurate photoelectric method (8). Similar studies of equal accuracy and precision should be made on chlorophyll in different solvents (lipoids, for instance) and in different physical states. Combined with measurements on living, green leaves and with fluorescence studies, this type of data would probably give some clear indications of the physical and chemical state of chlorophyll in the living cell.

The fluorescence of chlorophyll in certain solutions is very brilliant. This was first noticed by BREWSTER (1) in 1834 and it has been observed and studied by many investigators since that time, but like most observations on the fluorescence of organic compounds, these studies have been made chiefly by visual methods. DHERE (2), by the photographic method, found fluorescence maximima in ether solution at λ 6625 A. U. for chlorophyll *a*, and λ 6470 A. U. for chlorophyll *b*. The slit of his spectrograph was quite wide, viz., 0.25 mm., and therefore did not give good resolution. KAUTSKY and HIRSCH (3) have recently reported that the fluorescence intensity of leaves is correlated with the induction time of photosynthesis. Their experiments indicate that a detailed study of the fluorescent properties of chlorophyll would have important physiological significance.

From results of a quantitative spectral analysis of the fluorescence of chlorophyll, it is shown in this paper that it is possible to compare accurately the intensities of narrow regions of the fluorescence spectrum of chlorophyll. The spectro-photoelectric method described by ZSCHEILE, HOGNESS, and YOUNG (8) was used. This method is superior to that used by JETTE and WEST (5) because it permits a quantitative study of narrow regions of the fluorescence spectrum. They measured, by a photoelectric method, the damping of the total fluorescent radiation of the sodium salt of fluorescein by different ions in solution.

VALENTINER and ROSSIGER (4) showed that STOKE's law is not valid for fluorescein solutions and studied the energy yield of fluorescence as a function of the wave length of the exciting radiation. Similar studies, with improved technique, on a compound having the physiological importance of chlorophyll, would be extremely interesting.

The photometric apparatus was arranged as in Fig. 1. The round quartz fluorescence tube with one end tapered and blackened for a light trap according to the design used by R. W. WOOD, was placed next to the first monochromator slit, from which the guard window had been removed so that the origin of the fluorescent radiation might be as near the slit as possible. The light source was a 900 watt, 30 volt Mazda T-20 C clear projection lamp, operating at 675 watts on direct current from a generator supplemented by radio "A" batteries. The exciting light entered the tube at right angles to the collimator through a plane window. About 1 mm. from the window and just in front of slit 1 of the monochromator, it was brought to a focus. Ether solutions having a concentration of 0.015 g./l. of chlorophylls *a* and *b* were used. The components were prepared from fresh barley leaves by the method described by ZSCHEILE (7). This method is a combination and extension of the methods employed by WILLSTÄTTER and STOLL and by TSWETT. Fractionation between immisicible solvents provides a partial separation of the chlorophyll components. Chlorophyll *a* is finally purified by differential adsorption on talc and component *b* is purified by fractional precipitation from an ether-petroleum ether mixture.

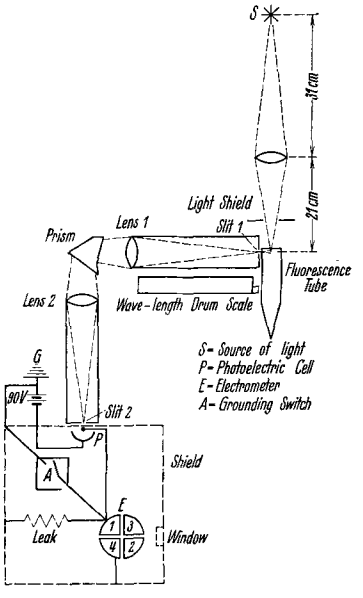

Fig. 1. Diagram of Apparatus

A Compton electrometer (with the high resistance leak of 1.65×10^{11} ohms), operating at a sensitivity of 10^{-15} amperes per mm. scale deflection, was used to measure the photoelectric current. The "dark current" was balanced out by a charge from a potentiometer circuit. The photoelectric cell used was the one whose sensitivity-wave-length curve was determined by YOUNG and PIERCE (6). The quadrant charge for each wave length, as measured by the electrometer deflections, was multiplied by the ratio of the thermopile deflection to the photocell deflection for the same wave length (from data of YOUNG and PIERCE) to obtain the fluorescence intensity-wave-length curve presented here. The monochromator slits were 0.03 mm. wide for chlorophyll *a* and 0.05 mm. wide for chlorophyll *b*. The widths of the spectral regions isolated by these slits varied from 35 A. U. at λ 6300 A. U. to 45 A. U. at λ 8200 A. U. for component *a* and from 55 A. U. at λ 6300 to 75 A. U. at λ 8200 A. U. for component *b*. Component *a* fluoresces more intensely under these conditions than does chlorophyll *b*.

The results are presented in Fig. 2, in which relative fluorescence intensity is plotted against wave length. Determinations were made at intervals of 25 A. U. over most of the region from λ 6300 to 8200 A. U. The writer has observed green fluorescence of chlorophyll solutions visually through a spectroscope but this region was not included in the present study. The maxima of the fluorescence bands are accurate to 10 A. U. Successive deflections for the same wave length may be repeated within 0.5 per cent. Maximum electrometer deflections of 26 centimeters, corresponding to a quadrant charge of 44 millivolts, were produced by the fluorescent light of λ 6690 A. U. from chlorophyll *a*.

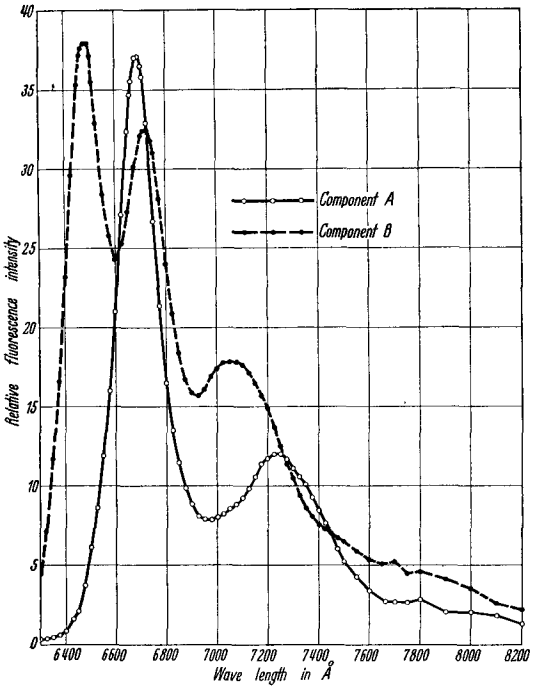

Fig. 2. Fluorescence Spectra of Chlorophylls *A* and *B* in Ether Solution

The fluorescence spectra of chlorophylls *a* and *b* in ether solution in the red region consist of well-defined continuous bands at room temperature. That of chlorophyll *a* has two maxima; at λ 6685 and 7230 A. U. That of component *b* has three maxima, at λ 6485, 6720, and 7050 A. U. Each component has a fluorescence band beyond 7000 A. U. in the near infra-red and fluoresces considerably as far as λ 8200 A. U. These curves are not comparable as to fluorescence intensity, since different slit widths were used in the two cases. Values on the same curve at different wave lengths are relative but no correction was applied for absorption of the fluorescent radiation by chlorophyll before it reached the boundary of the solution. Care was taken to have the point of origin of the fluorescence as near as possible to the edge of the fluorescence tube and the slit. The ratios of the maxima of either curve have an accuracy of only 5 per cent, due to photodecomposition of the chlorophyll during the measurements.

This method of spectral measurement appears suitable for the study of many factors affecting the fluorescence of chlorophyll, such as (1) solvent, (2) concentration, (3) temperature, (4) spectral quality of exciting radiation (STOKE's law), (5) state of dispersion of chlorophyll or its solvent in colloidal systems, (6) presence and activity of possible damping agents, as carbon dioxide, oxygen, water, carotene, xanthophyll, proteins, lipoids, carbohydrates, and other compounds found associated with chlorophyll in the green plant cell, and (7) the effect of one component of chlorophyll on the fluorescence of another. The

variation of fluorescence intensity with that of the exciting radiation would indicate the number of quanta of energy required to excite the chlorophyll molecule to different energy states. When such problems have been effectively studied, perhaps we shall know more concerning the detailed kinetics and energetics of the photosynthetic reaction.

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