THE CHLOROPHYLL UNIT IN PHOTOSYNTHESIS

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Emerson and Arnold (1) have shown that for each molecule of carbon dioxide reduced per flash of light by the green alga *Chlorella pyrenoidosa*, approximately 2,500 molecules of chlorophyll are present.[1] This suggests the existence of a chlorophyll unit in the photosynthetic mechanism. Before such a suggestion may be accepted, however, at least four possible criticisms must be met:

I. There may be a large error in the determination of the oxygen production by means of the Warburg-Barcroft apparatus.

II. There may be a large error in the determination of the chlorophyll content by means of the spectrophotometer.

III. *Chlorella pyrenoidosa* may be unique in having such a large ratio.

IV. The theory upon which rests the analysis of photosynthesis by means of flashing light (2) may be inadequate.

The purpose of this paper is to discover whether any of the foregoing forbids the assumption of a chlorophyll unit.

I

To check the accuracy of the manometric determinations, oxygen was produced by electrolysis in a specially adapted Warburg vessel at a rate approximately equal to that obtained in experiments on photosynthesis. It was found that the rate determined manometrically agreed to within 10 per cent with that calculated by Faraday's laws. These experiments were performed with Mr. C. S. French.

[1] The conditions of their experiments were such that the cells, suspended in carbonate buffer, were illuminated intermittently by short flashes (less than 1×10^{-4} sec.), separated by dark periods adequate for the restitution of the photochemical reaction to maximum sensitivity. Light saturation and carbon dioxide saturation were maintained.

II

The spectrophotometric estimation of chlorophyll was checked by determining the value of the extinction coefficient of a standard solution, containing 10 mg. of chlorophyll per liter of methanol, according to the method previously described (1). Two monochromatic sources were used, neon—6598 Å., and helium—6678.15 Å., of which the latter can be made the brighter. The chlorophyll preparations, from horseweed and spinach, had been extracted in Professor O. L. Inman's laboratory at Antioch College, and were obtained through the courtesy of Dr. Emma M. Dietz. In Table I the values of the extinction coefficient obtained are compared with the value previously published by Emerson and Arnold (1). No significant differences are apparent.

TABLE I

Extinction Coefficients for Methyl Alcohol Solutions Containing 10 Mg. of Chlorophyll per Liter

Chlorophyll solutions	Neon —6598.95 Å.	Helium —6678.15 Å.
Chlorella (Emerson and Arnold (1))	0.476	—
Spinach	0.485	0.449
Horseweed	0.486	0.459

For the preparations of chlorophyll used above, the value of the ratio of chlorophyll *a* to chlorophyll *b* is about 3 to 1. Since approximately this value holds for most plant material, the extinction coefficients determined may be generally applied. The chlorophyll estimations thus made will not be absolute, but such errors as are resident in them cannot decrease markedly the computed size of the chlorophyll unit.

III

Since the high value of the ratio, mols of chlorophyll/mols of carbon dioxide reduced per flash, may be a unique property of *C. pyrenoidosa*, we have determined its value for six species of plants drawn from four phyla, by experimental methods similar to those utilized by Emerson and Arnold (1). The results, summarized in Table II, indicate

that the value of the ratio, designated ρ, normally falls between 2,000 and 4,000. Adverse culture conditions and ultraviolet radiation can

TABLE II

Values of the Ratio ρ, Mols of Chlorophyll/Mols of Carbon Dioxide Reduced per Flash, for Six Species of Plants

Plant	Position of material in respirometer vessel	Temperature	Ratio
		°C.	
Bryophyllum calycinum			
1	Leaf floated on surface of carbonate buffer*	31.5	2,500
2	" "	31.5	2,600
Chlorella vulgaris	Suspended in buffer	25.0	2,800
Lemna sp.			
1	Floated in buffer	25.0	2,600
2	" " "	25.9	3,200
3	" " "	29.1	2,900
Nicotina langsdorffii (flowers)			
1	Supported upright in well partly filled with tap water; surrounding air space partly filled with buffer	29.9	2,800
2		29.2	2,500
Selaginella sp.	Stem in tap water in side arm; leaves projected above buffer in air space	28.3	4,200
Stichococcus bacillaris			
1	Suspended in buffer	25.0	3,700
2	" " "	29.7	5,000

* Carbonate buffer composed of 15 parts 0.1 M K_2CO_3 plus 85 parts 0.1 M $KHCO_3$.

increase the value of ρ, but in no case have we been able to decrease the value to a small number.

IV

The significance of the high value of ρ, that a large number of chlorophyll molecules are present for each molecule of carbon dioxide reduced, is dependent upon the validity of the assumptions made in the analysis of photosynthesis under flashing light (2). The most important of these, for our present purposes, is that which dictates the availability of all chlorophyll for photochemical work at the beginning of each flash. However, if the life-time of the chlorophyll reaction should extend over the period occupied by a sufficient number of flashes and their accompanying dark-times, the value of ρ would become a small number; *e.g.*, 10. But this possibility is rendered unlikely by the experiments of Warburg (3), who found the light efficiency of photosynthesis in *Chlorella* to be well over 50 per cent. Since the efficiency is high, a life-time long enough to reduce the value of ρ would imply so large a change in the absorption coefficient of chlorophyll during photosynthesis that it could not have escaped detection.

SUMMARY

In six species of plants, representing four phyla, the minimum number of chlorophyll molecules present for each molecule of carbon dioxide reduced appears to lie between 2,000 and 3,000. This finding suggests the existence of a chlorophyll unit.

CITATIONS

1. Emerson, R., and Arnold, W., *J. Gen. Physiol.*, 1932–33, **16**, 191.
2. Emerson, R., and Arnold, W., *J. Gen. Physiol.*, 1931–32, **15**, 391.
3. Warburg, O., and Negelein, E., *Z. phys. Chem.*, 1923, **106**, 191.