

PHOTOSYNTHETIC EFFICIENCY OF MARINE PLANTS*

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All photosynthetic organisms contain accessory pigments which in many cases may account for a large fraction of the total light absorbed. As a result of this the question of the role of these pigments in photosynthesis is significant. Of the methods described for the estimation of accessory pigment activity, only a measure of the quantum efficiency as a function of wave length or detailed action spectra are considered to be satisfactory. Results of the first type of experiments have led to the conclusion that the carotenoids are generally partially active (14, 15, 19, 33, 39, 44) while the carotenoid pigment fucoxanthol (8, 40, 47) and the phycobilin pigment phycocyanin (14) operate with a quantum efficiency approaching that of chlorophyll itself. Action spectra of fluorescence (9, 10, 20) and phototaxis (32, 41), both of which are assumed to represent photosynthesis, have also indicated that there is partial carotenoid activity in green plant and bacterial photosyntheses.¹ In polarographic studies of photosynthetic action spectra of multicellular marine algae, Haxo and Blinks (22) observed carotenoids of *Ulva*, a green alga, and of *Coilodesme*, a brown alga, to be only slightly less efficient than chlorophyll. Entirely unexpected results were obtained with a number of red algae. Here the most active pigments were phycoerythrin and phycocyanin while the least active were chlorophyll and carotenoids. Duysens (10), on the basis of action spectra of photosynthesis and of chlorophyll fluorescence, drew similar conclusions for the unicellular red alga *Porphyridium*. The problems associated with the accessory pigments have recently been reviewed (3, 4).

In the present investigation, the action spectra mentioned above (22) have been extended by measurements of quantum efficiency of photosynthesis in representative marine algae, one arboreal green alga, and one marine flowering plant. The results and interpretations are in agreement with those previously reported.

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¹ Hoover's results (25) likewise indicate some participation of carotenoids in the photosynthesis of wheat seedlings.

Materials and Methods

In general the plant materials and the methods of Haxo and Blinks (22) have been employed with the exception that the polarographic method for following the rate of photosynthesis was replaced by methods permitting computation of absolute rates.

1. Incident Radiation.—

The monochromator was modified slightly as follows. The lamp was replaced by an entrance slit illuminated with a similar lamp and condensing lenses. Either an image of the grating or an image of the exit slit 2×15 to 20 mm. was brought to the focal plane of the algae with a pair of achromatic lenses and a totally reflecting prism. By rotating the prism the incident beam was directed into the thermopile for incident energy measurements or into the integrating sphere (or hemisphere) for measurements of light absorption by the algae. The thermopile was calibrated with a standard carbon filament lamp operated in accordance with methods described by the National Bureau of Standards. The observed incident energy values were multiplied by 0.935, the thermopile window transmission ratio for standard lamp radiation/visible radiation. Appropriate corrections for reflection losses in the experimental light path were also made. In cases in which the average intensity throughout the usable light field was different by more than 3 per cent from that in the area covered by the thermopile, an experimentally determined correction was applied. In most cases five wave length regions of band half width 15 $m\mu$ were used. They were selected on the basis of known absorption maxima of the various plastid pigments:

- 675 $m\mu$, chlorophyll a
- 620 " , phycocyanin ("C-phycocyanin")
- 560 " , phycoerythrin
- 500 " , carotenoids
- 436 " , carotenoids and chlorophyll

The glass filters used by Haxo and Blinks for removal of stray light were omitted.

2. Absorbed Radiation.—

That fraction of the incident radiation absorbed by the algae was determined by measurement of the transmitted plus reflected (scattered) fraction and subtracting them from one. The integrating hemisphere method described by Haxo and Blinks was used. In order to test this technique for the introduction of possible errors, it was compared to the more generally used integrating sphere method (27, 35, 37) originally described by Ulbricht. When absorption values were greater than 50 per cent, agreement between the two methods within 5 per cent of the absorption values themselves was obtained. Absorption by thalli, even in wave length regions of absorption minima, was increased to 50 per cent or higher by backing the thalli with white vinylite sheeting. No detectable changes in thallus absorption caused by light were observed.

The optical paths for the measurement of incident energy, absorbed energy, and photosynthesis were identical except for small reflection and negligible absorption losses.

3. Photosynthesis Measurements.—

Of the several methods employed for measurement of photosynthesis only that which was the finally adopted, namely the Fenn (17) type differential volumeter, will be described. An experimental vessel 1.5 mm. deep and 35 mm. in diameter was machined of lucite. Fig. 1 is a diagram in cross-section. The vessel bottom 0.5 mm. deep contained the "liquid" phase, a 0.38 mm. layer of 2 to 4 per cent gelatin. The algal thalli adhered to the vessel top 1 mm. above the liquid phase. Mixing within the vessel was by diffusion. The vessel was connected to a straight horizontal pyrex capillary 0.50 mm. inside diameter, containing an index drop of triply distilled kerosene.

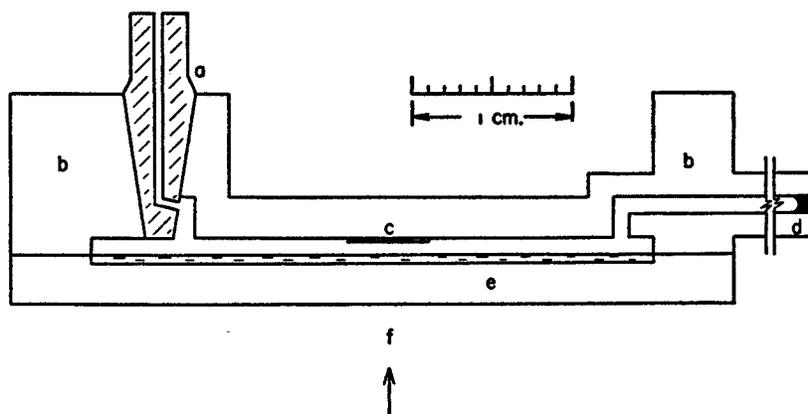


FIG. 1. Diagram of the experimental vessel in section through the axis of the capillary. Vent plug, *a*; vessel top, *b*, with adhering algal thallus, *c*, and attached capillary, *d*; vessel bottom, *e*, containing liquid phase. The two portions of the vessel are held together with a pair of metal rings drawn tight with screws. The light beam, *f*, comes from below and passes through the vessel bottom.

The other end of the capillary was connected through a 3-way stop-cock to a coil of 5 mm. inside diameter glass tubing 75 cm. long which served as a compensating vessel. After loading and closing, the entire apparatus was submerged into an insulated lucite water bath. In order to minimize variable temperature differentials between the experimental and compensating vessels that result from conventional on-off thermoregulators, no device of this type at all was used. Instead the bath was filled with water at 15°C. and stirred. During the course of an experiment the temperature never increased by more than 1°C. An equation for converting distance traversed by the index fluid into gas exchange is derived in the Appendix.

In most of the experiments oxygen exchange was measured while maintaining a constant partial pressure of 0.02 per cent CO₂. For this purpose the gelatin liquid phase was equilibrated in 0.3 M Na₂CO₃ + 0.1 M NaHCO₃ prior to the experiment. This mixture is isotonic with sea water and liberated CO₂ at a rate which supported photosynthesis at least 6 times the maximal observed rate of photosynthesis.

A few experiments were carried out in 5 per cent CO₂ employing the two-vessel

principle of Warburg (42). Rate measurements were made first with the vessel containing 0.385 ml. gelatin buffer, $M/2$ NaCl, and $M/20$ KH_2PO_4 . Duplicate measurements were then made after the gelatin buffer had been removed.

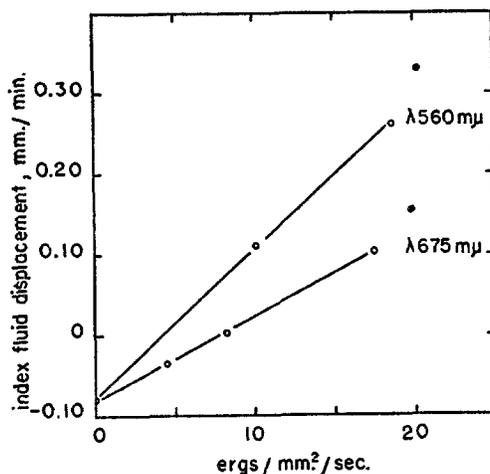


FIG. 2. Rate of *Porphyra nereocystis* photosynthesis as a function of light intensity. Quantum efficiencies may be computed from the experimental points and the following additional information. Vessel factor (5.52×10^{16} molecules of oxygen per mm. index fluid displacement). Thallus area (132 mm.^2).

Wave length, $m\mu$	Thallus absorption	Quanta per erg
560	0.70	2.86×10^{11}
675	0.60	3.44×10^{11}

The computed efficiencies are as follows:—

Wave length, $m\mu$	Solid circles	Open circles
560	0.070	0.064
675	0.040	0.034

Data indicated by solid circles were obtained first. After replacing and calibrating the lamp the data indicated by open circles were then obtained.

After the vessel was loaded and placed in the water bath the algae were illuminated for about one-half hour at several times the compensating intensity. Then the position of the index fluid was observed to the nearest 0.01 mm. every minute during alternate 10 minute light and dark intervals. Rates of photosynthesis were computed from the steady state rate of gas exchange during the last 5 minutes of these intervals. In some cases the steady state in 5 per cent CO_2 was established only after longer (up to 15 minutes) time periods. The rate of respiration in the light was assumed to be equal to that in the dark.

RESULTS

Efficiency as a Function of Experimental Variables

Certain preliminary experiments were carried out to examine the relation between quantum efficiency and various parameters under control during the experiments. They were altered one at a time while all other variables were maintained at values approximately equal to those of the final experiments. The efficiency was found to be equal within 3 per cent at 12 and 19°C., but at 30°C. the efficiency was reduced by about 20 per cent. All subsequent experiments were carried out between 11 and 19°C. Increasing the CO₂ partial pressure from 0.02 to 0.05 per cent or variations of the light intensity between 3 and 30

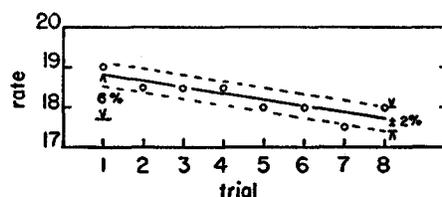


FIG. 3. A series of rate measurements computed from alternate 10 minute dark and light periods, the latter at constant wave length and intensity. The rates are expressed as index fluid displacement in hundredths of mm./min. and are plotted in the order in which they were obtained.

ergs/mm.²/sec. altered the efficiency by negligible quantities. Slightly lower efficiencies were observed in the region of 60 ergs/mm.²/sec. Typical light curves are presented in Fig. 2. They show a nearly linear dependence of photosynthetic rate upon light intensity and pass through the origin when extrapolated to zero intensity. No departures from linearity in the region of the compensation point (26–28) were ever noted. Repeated measurements on the same plant material (Fig. 3) showed satisfactory agreement, with a small decline in rate with time. This decrease in rate was tested for in each experiment but in most cases was negligible. It was concluded that the observed efficiencies were not appreciably limited by experimentally controllable parameters during the course of the experiments. The accuracy of the final results was probably limited largely by the photometric measurements. Other possible systematic errors such as inadequate buffering by the carbonate, non-uniformity of capillary bore, and decrease in effective bore of the capillary by wetting with index fluid were insignificant, their sum altering the final values less than 1 per cent.

Quantum Efficiency as a Function of Wave Length

(a) *One-Vessel Principle, 0.02 Per Cent CO₂.*—Results of some experiments with several green algae are presented in Table I. Although the results appear to be somewhat variable the following trends were noted. In *Ulva* values of

approximately 0.07 were obtained at λ 675, 620, and 436 $m\mu$. The apparent efficiencies at λ 500 and 560 $m\mu$ are more variable and generally lower. The data from an experiment with *Monostroma* show that when the value at λ 675 $m\mu$ is low (0.05) it is equally low for the remainder of the wave length regions.

Data obtained from brown algae are presented in Table II. Within each experiment on a single thallus sample, a rather uniform quantum efficiency with respect to wave length was observed. The absolute values, however, for

TABLE I
Quantum Efficiency of Green Algae at Different Wave Lengths of Incident Radiation

Alga	Wave length, $m\mu$				
	436	500	560	620	675
<i>Monostroma zostericola</i>	0.041	0.044	0.044	0.041	0.051
<i>Ulva lobata</i>	0.070	0.055	0.043	0.061	0.080
	0.066	0.048	0.050	0.060	0.078
<i>Ulva lactuca</i>	0.062	0.071	0.073	0.067	0.068
	0.074	0.054	0.064	0.064	0.064

TABLE II
Quantum Efficiency of Brown Algae at Different Wave Lengths of Incident Radiation

Alga	Wave length, $m\mu$				
	436	500	560	620	675
<i>Coilodesme californica</i>	0.056	0.054	0.056	0.058	0.051
	0.069	0.073	0.070	0.076	0.075
<i>Ilea fascia</i> (a) young thallus	0.097	0.085	0.086	0.090	0.092
	0.073	0.067	0.063	0.076	0.087

each experiment vary over a wide range from 0.05 to 0.09. The data for the "old" *Ilea* thallus are exceptional in that somewhat lower efficiencies were found at λ 500 and 560 $m\mu$ than at the other three wave length regions.

Table III contains data derived from experiments with red algae. With minor exceptions, approximately equal quantum efficiencies of 0.07 obtain at λ 500, 560, and 620 $m\mu$. The values at λ 675 $m\mu$ are lower by one-third to one-half while those at λ 436 $m\mu$ were lower by one-half or greater. Essentially the same results were obtained from at least one representative species from each of the seven classes in the Monterey Bay region Rhodophyceae.

Quantum efficiencies of an arboreal green alga and one marine flowering

plant are included in Table IV. *Trentepohlia* was extremely inefficient at all the shorter wave length regions while *Phyllospadix* leaves were approximately as efficient as *Ulva* (Table I) at all the five wave length regions investigated.

(b) *Two-Vessel Principle, 5 Per Cent CO₂*.—In Table V are listed the results

TABLE III
Quantum Efficiencies of Red Algae at Different Wave Lengths of Incident Radiation

Alga	Wave lengths, $m\mu$				
	436	500	560	620	675
<i>Porphyra nereocystis</i> (Bangioideae)	0.021	0.058	0.072	0.069	0.043
	0.037	0.062	0.068	0.065	0.035
		0.051	0.057	0.052	0.025
			0.078		0.038
<i>Porphyra naiadum</i> (Bangioideae)	0.028	0.042	0.059	0.056	0.040
	0.021	0.058	0.072	0.069	0.043
			0.075		
<i>Delesseria decipiens</i> (Florideae)	0.034	0.063	0.063	0.052	0.031

TABLE IV
Quantum Efficiencies of *Trentepohlia* sp. and *Phyllospadix* Leaves

Plant	Wave length, $m\mu$				
	436	500	560	620	675
<i>Trentepohlia</i> (green alga)	<0.001	<0.001	0.009	0.024	0.054
<i>Phyllospadix scouleri</i> (flowering plant)	0.059	0.048	0.056	0.068	0.075 0.073

TABLE V
Quantum Efficiencies of Two Red Algae Determined by the Two-Vessel Principle

Alga	Wave length, $m\mu$	ϕ_{O_2}	$\gamma = \frac{CO_2}{O_2}$
<i>Porphyra nereocystis</i>	436	0.066	-0.95
	500	0.089	-0.95
	560	0.101	-0.90
	620	0.105	-0.94
	675	0.046	-0.96
<i>Aeodes gardneri</i>	560	0.092	-0.97
	675	0.035	-0.87

obtained with the two-vessel principle for two red algae. The computed efficiencies, although higher than those generally obtained with the one-vessel method with carbonate buffer, show the characteristic dependence upon wave length (*cf.* Table III). The photosynthetic quotient was close to one and independent of wave length. In a direct comparison of the two-vessel with the one-vessel method the following results were obtained for the same *Ulva* thallus disc:—

One-vessel, carbonate buffer, $\phi_{o_2} = 0.075$

Two-vessel, 5 per cent CO₂, $\phi_{o_2} = 0.077$, $\gamma = - 0.95$

In the experiments carried out in 5 per cent CO₂, volume “bursts,” similar to the pressure “bursts” recorded by Emerson and Lewis (12, 13) for *Chlorella*, were consistently observed during the initial 5 to 15 minutes of both light and

TABLE VI
Quantum Efficiencies of Porphyra naiadum and Ulva lobata Collected from the Same Ecological Location

Mean, \bar{x} , and standard deviation, s , of seven collections.

Alga	Wave length, $m\mu$	\bar{x}	s
<i>Porphyra naiadum</i>	560	0.066	0.005
	620	0.064	0.009
	675	0.031	0.007
<i>Ulva lobata</i>	675	0.069	0.005

dark periods. These rapidly changing rates along with other uncertainties in practice of the two volume method made determination of rates less accurate than the determination of those in 0.02 per cent CO₂ in which no volume “bursts” were noted.

(c) *Comparative Experiments.*—In order to reduce the experimental and ecological factors that might account for variable and low efficiencies, the relative quantum efficiencies of naturally associated marine alga were compared under more similar experimental conditions than those reported above in Tables I to IV. Thalli of the red alga *Porphyra naiadum* and the green alga *Ulva lobata* were collected from within 1 foot of each other every day for 7 days during tidal cycles that precluded illumination of the exposed algae by direct sunlight. Comparisons of photosynthetic rates were then made as soon as possible and were complete within several hours. For these experiments the algae were suspended in sea water in a differential volumeter similar to that described by Fenn (17). As a result of the incomplete CO₂ buffering by the sea-

water (11), the rate of gas exchange was multiplied by 1.3, an experimentally determined factor, to obtain the rate of oxygen exchange. The results (Table VI) when tested for equality of means by Hoel's (24) method for small samples, show no significant differences between the *Porphyra* efficiencies at $\lambda 560$ and $620\text{ m}\mu$ or between these and those of *Ulva* at $\lambda 675\text{ m}\mu$. The $\lambda 675\text{ m}\mu$ value for *Porphyra* is lower by one-half than the above three mentioned ones, and is highly significant.

DISCUSSION

In view of the inherent experimental difficulties, it seems doubtful that the results presented here represent the quantum efficiency of marine plants to an accuracy any greater than 10 to 20 per cent. Within these limits, it has been concluded that the maximal efficiency of representative green (Tables I and VI), brown (Table II), and red (Tables III and VI) algae, and one flowering plant (Table IV) is equal to 0.08 molecule of oxygen liberated per absorbed quantum. This corresponds to a conversion of 20 to 25 per cent, depending upon the wave length, of the energy available as quanta into potential chemical energy. That the highest values of 0.10 were obtained in 5 per cent CO_2 is probably without significance, and the result of possible interference by the volume "bursts" and other uncertainties of the two-vessel method previously mentioned.

Now that estimates of the quantum efficiency of red algae (Rhodophyceae) and brown algae (Phaeophyceae) are available it is possible to make comparisons among all major groups of photosynthetic organisms. The maximum values reported here are similar to those of leaves of higher plants (1, 21, 45) as well as those of laboratory cultured unicellular algae (1, 8, 12-15, 33, 34, 37, 40), "wild" diatoms (46), and bacteria (16, 18, 29); see Rabinowitch (36) for a review. It is tentatively concluded that the maximum quantum efficiency of all organisms is essentially equal and this is interpreted to mean that the limiting mechanisms are similar in all these cases. Our results are in disagreement with the much higher values of 0.25 obtained by Warburg and collaborators for *Chlorella* in 5 per cent CO_2 (43, 44).

An estimate of the quantum efficiency of photosynthesis sensitized by each of the accessory pigments themselves was computed from the efficiency of the thalli and estimates of the fractional absorption by the various components. The method was similar to those methods previously described (2, 9) except that the isolated components providing the absorption data were not obtained from the same plant material which furnished the thallus absorption and efficiency data. Table VII contains the results of portional absorption calculations for the red alga *Porphyra naiadum*. At $\lambda 675\text{ m}\mu$ the major absorption is that of chlorophyll *a* (see below). At $\lambda 620\text{ m}\mu$ approximately one-third of the absorp-

tion is by chlorophyll and two-thirds by phycocyanin. At $\lambda 560\text{ m}\mu$ a large fraction (*ca.* 84 per cent) is absorbed by phycocyanin and phycoerythrin, the latter absorbing 67 per cent. The precision of these values is considered to be rather low largely because of the substitution of blue-green algal chlorophyll protein for that of a red alga. This procedure was adopted because red algal chlorophyll proteins suitable for spectrophotometric analysis have not yet been prepared. From the data of Table VII and the average quantum efficiency of *Porphyra*

TABLE VII

Fractional Absorption by Chromoproteins in Porphyra naiadum

Optical densities of the components were multiplied by arbitrary concentration factors so that their sum was approximately equal to the thallus optical density at $\lambda 675, 620,$ and $560\text{ m}\mu$. Negligible absorption by other cellular components was assumed.

	Wave length, $m\mu$				
	436	500	560	620	675
	Optical densities				
1. Thallus.....	0.96	0.64	0.64	0.48	0.64
2. Chlorophyll protein*	0.89	0.24	0.10	0.17	0.64
3. Phycocyanin†	0.00	0.01	0.11	0.30	0.00
4. Phycoerythrin†	0.01	0.19	0.43	0.00	0.00
5. Sum of 2, 3, and 4.....	0.90	0.44	0.64	0.47	0.64
	Per cent absorption (approximate)				
6. Chlorophyll plus carotenoids.....	0.95		16	36	100
7. Phycocyanin.....			17	64	0
8. Phycoerythrin.....			67	0	0
9. Sum of 7 and 8.....			84	64	0

* Chlorophyll *a*, carotenoid protein from the blue green alga *Tolypothrix lanata* (38).

† Phycocyanin and phycoerythrin isolated from *Porphyra naiadum* and purified by chromatography (23).

naiadum photosynthesis in Table III, the efficiency by which each pigment sensitizes photosynthesis was estimated:—

Chlorophyll *a*, 0.042
Phycocyanin, 0.075
Phycoerythrin, 0.073

The values for phycocyanin and phycoerythrin are in agreement with the value calculated for phycocyanin of *Chroococcus* (2). It is concluded that the phycobilin pigments of red algae sensitize photosynthesis with a quantum efficiency approaching that of chlorophyll in *Chroococcus*, the green and brown algae, and about twice that of chlorophyll in red algae.

By similar reasoning the data for the green algae *Ulva* and *Monostroma* (Table I) and the brown algae *Coilodesme* and *Ilea* (Table II) support the conclusion of Haxo and Blinks (22) that the carotenoid pigments of the former are mostly available for photosynthesis while the efficiency of fucoxanthol of the latter is nearly as great as that of chlorophyll on an absorbed quantum basis. Brown algae are, therefore, photosynthetically very similar to the diatoms (8, 40, 47). The variable but generally lower efficiency of red algae at $\lambda 436 \text{ m}\mu$ (Table III) in which about one-half the total light absorbed is due to carotenoids provides supporting evidence that these pigments are here largely inactive in photosynthesis (10, 22). Quantum efficiency of the arboreal green alga *Trentepohlia* (Table IV) diminishes to very low values toward the shorter wave length regions. Here the inactive pigments are carotenoids localized in oil droplets and present in large concentrations. The carotenoid: chlorophyll ratio in *Trentepohlia* was found to be 50 times that of *Ulva*.

Until now in the present discussion it has been assumed that the relatively low quantum efficiency of red algae in the red and blue portions of the spectrum is due to chlorophyll being inefficient. A second possibility is that some other pigment, inactive in photosynthesis, absorbs about one-half the incident radiation at these wave lengths. All the available evidence supports the former view. The chlorophyll *d* content of *Porphyra* was found to be so low that it can account for less than 0.1 per cent of the light absorbed in the wave length region of the red chlorophyll *a* maximum. Absorption spectra of methanol extracts from a completely extracted *Porphyra* thallus showed absorption only by chlorophyll *a* and no absorption bands at $\lambda 675 \text{ m}\mu$ remain in the extracted thallus. This is in agreement with Manning and Strain's observations (31) that red algae contain only chlorophylls *a* and *d*. Finally the absorption spectra of extracts from "adapted" thalli, the efficiency of which had been increased experimentally by exposure to red or blue light for several days (48), were indistinguishable from those of freshly collected algae. It was concluded that (*a*) the inactive pigment of red algae collected from their natural habitat is chlorophyll *a*, or that (*b*) a portion of the chlorophyll sensitizes a partial (photooxidation) or complete (respiration) reversal of the normal photosynthesis.

If photooxidation were responsible there might be a larger photosynthetic quotient (CO_2/O_2) in red and blue light than in green light as most photooxidations absorb oxygen without the liberation of carbon dioxide (5). Photosynthetic quotients close to 1.0 and independent of radiation wave lengths were always observed (Table V). Also when a glass electrode was used to measure CO_2 exchange by changes in pH, the results, as a function of wave length, were very similar to those obtained for oxygen. An increase in respiration sensitized by chlorophyll and large enough to account for the low efficiencies should result in a lower apparent saturation rate in red and blue light than in green light. Some volumetric and polarometric experiments to test this hy-

pothesis showed that the maximum steady state rate of photosynthesis in excess CO_2 was independent of the wave length of the incident radiation. It was also independent of wave length in the presence of inhibiting concentration of hydroxylamine or dinitrophenol, reagents which inhibit close to the photochemical process (30). When red light ($> \lambda 660 \text{ m}\mu$) of saturating intensity was rapidly substituted by green light of saturating intensity, and *vice versa*, no induction phase was apparent. These experiments were interpreted to mean that (a) a single series of processes limits the maximal rate of photosynthesis in red algae in excess CO_2 , (b) this rate-limiting process is independent of the light-absorbing pigment, and (c) chlorophyll *a* is not sensitizing a back reaction such as respiration. This leads to the conclusion that the probability of each chlorophyll molecule operating at maximal efficiency is 0.5 or less; or that there are two kinds of chlorophyll, the first with a probability approaching 1, and the second approaching 0.

A choice between the two alternatives is possible with the aid of a knowledge of the mechanism of phycobilin-sensitized photosynthesis. From studies of red algal fluorescence spectra as a function of wave length of incident radiation, Duysens (9, 10), and French and Young (20) have shown that light energy absorbed by phycoerythrin is transferred to phycocyanin and from phycocyanin to chlorophyll by resonance transfer. This process can also account for phycocyanin-sensitized photosynthesis in the blue green alga *Chroococcus* (Arnold and Oppenheimer (2)). In connection with the present investigation some preliminary quantitative measurements of time course changes of fluorescence during the induction phase were made and these also show a transfer of excitation energy from phycobilin pigments to chlorophyll. But the quantum efficiency experiments (Table III) show that phycobilin photosynthesis is twice as efficient as that of chlorophyll and there remains the problem of how an efficient process can proceed *via* molecules the whole population of which is far less efficient when excited by light directly. The results of the action spectra, quantum efficiency, and fluorescence experiments can be accounted for by assuming that the chlorophyll is divided about equally into active (in both photosynthesis and fluorescence) and inactive molecules, with the phycobilins transferring energy almost entirely to the active ones. This, apparently, would demand a high degree of organization within the plastid rather than a random distribution of pigments; a high probability of a phycocyanin molecule exciting an active chlorophyll and a low probability of exciting an inactive molecule. The probability of an active chlorophyll exciting an inactive one would necessarily be low also.

SUMMARY

Multicellular marine plants were collected from their natural habitats and the quantum efficiency of their photosynthesis was determined in the laboratory

in five narrow wave length bands in the visible spectrum. The results along with estimates of the relative absorption by the various plastid pigments show a fairly uniform efficiency of 0.08 molecules O_2 per absorbed quantum for (a) chlorophyll of one flowering plant, green algae, and brown algae, (b) fucoxanthol and other carotenoids of brown algae, and (c) the phycobilin pigments phycocyanin and phycoerythrin of red algae. The carotenoids of green algae are sometimes less efficient while those of red algae are largely or entirely inactive. Chlorophyll *a* of red algae is about one-half as efficient ($\phi_{o_2} = 0.04$) as either the phycobilins, or the chlorophyll of most other plants. These results as well as those of high intensity and of fluorescence experiments are consistent with a mechanism in which about half the chlorophyll is inactive while the other half is fully active and is an intermediate in phycoerythrin- and phycocyanin-sensitized photosynthesis.

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APPENDIX

Although the theory of the differential volumeter has been developed by several investigators, a single exact equation relating traverse of the index fluid to volume of gas exchange has not yet come to the authors' attention. Fenn (17) assumed that both experimental and compensating vessels contained the exchanging gas only and later applied a correction for solubility of this gas in a liquid phase. Cunningham and Kirk (6) corrected for solubility of both exchanging and non-exchanging gases while Dixon (7) made incomplete corrections of the exchanging gas only.

Derivation of an equation for a differential volumeter requiring neither the above-mentioned assumption nor the assumption that the volume of the vessels is large compared to that of the exchanging gas (6, 17) is as follows:—

Let

- (a) A = cross-section of capillary.
- (b) F = vessel factor for converting distance traversed by the index drop to gas volume at standard temperature and pressure.
- (c) h = distance travelled by index drop after adding x μ l. O_2 at s.t.p. to the experimental vessel.
- (d) $p_{CO_2}^c, p_{O_2}^c, p_{N_2}^c$ = partial pressure in atmospheres, of CO_2 , O_2 , and N_2 respectively, in the experimental vessel. The p values were computed from the composition of the water vapor-saturated gas mixture flushed through the vessel.
- (e) $p_{CO_2}^e, p_{O_2}^e, p_{N_2}^e$ = as in (d) except after addition of x μ l. at s.t.p. of oxygen to the experimental vessel.
- (f) $p_{CO_2}^c, p_{O_2}^c, p_{N_2}^c, p_{CO_2}^e, p_{O_2}^e, p_{N_2}^e$ as in (d) and (e) except for the compensating vessel.

- (g) V_o^e, V_f^e = gas and liquid volume respectively of the experimental vessel.
 (h) V_o^c, V_f^c = gas and liquid volume respectively of the compensating vessel.
 (i) $\alpha_{CO_2}, \alpha_{O_2}, \alpha_{N_2}$ = solubility coefficients for $CO_2, O_2,$ and N_2 respectively, in volumes of gas at s.t.p. dissolved in one volume of liquid at temperature T and a gas pressure of one atmosphere.
 (j) T = absolute temperature of experimental and compensating vessels.
 (k) y = vapor pressure, in atmospheres, of water at temperature T . y is equal in the two vessels since they both contain the same buffer solution. Lowering of the vapor pressure by the presence of salts reduces y by 1 per cent and has been neglected.

When x is added to the experimental vessel the index drop is displaced by a volume equal to Ah and the pressure in the entire system rises. In the experimental vessel:—

$$p_{O_2}^e \left(V_o^e \frac{273}{T} + \alpha_{O_2} V_f^e \right) + x = p_{O_2}^c \left(V_o^c \frac{273}{T} + Ah \frac{273}{T} + \alpha_{O_2} V_f^c \right) \quad (1)$$

$$p_{N_2}^e \left(V_o^e \frac{273}{T} + \alpha_{N_2} V_f^e \right) = p_{N_2}^c \left(V_o^c \frac{273}{T} + Ah \frac{273}{T} + \alpha_{N_2} V_f^c \right) \quad (2)$$

$$p_{CO_2}^e \left(V_o^e \frac{273}{T} + \alpha_{CO_2} V_f^e \right) = p_{CO_2}^c \left(V_o^c \frac{273}{T} + Ah \frac{273}{T} + \alpha_{CO_2} V_f^c \right) \quad (3)$$

$$p_{O_2}^e \left(V_o^e \frac{273}{T} + \alpha_{O_2} V_f^e \right) = p_{O_2}^c \left(V_o^c \frac{273}{T} - Ah \frac{273}{T} + \alpha_{O_2} V_f^c \right) \quad (4)$$

$$p_{N_2}^e \left(V_o^e \frac{273}{T} + \alpha_{N_2} V_f^e \right) = p_{N_2}^c \left(V_o^c \frac{273}{T} - Ah \frac{273}{T} + \alpha_{N_2} V_f^c \right) \quad (5)$$

$$p_{CO_2}^e \left(V_o^e \frac{273}{T} + \alpha_{CO_2} V_f^e \right) = p_{CO_2}^c \left(V_o^c \frac{273}{T} - Ah \frac{273}{T} + \alpha_{CO_2} V_f^c \right) \quad (6)$$

Solving for x by rearranging equation 1:

$$x = p_{O_2}^c \left(V_o^c \frac{273}{T} + Ah \frac{273}{T} + \alpha_{O_2} V_f^c \right) - p_{O_2}^e \left(V_o^e \frac{273}{T} + \alpha_{O_2} V_f^e \right) \quad (7)$$

Only $p_{O_2}^c$ in equation 7 is unknown and may be solved for as follows. The pressures in the experimental and compensating vessels are always equal and

$$p_{O_2}^e + p_{N_2}^e + p_{CO_2}^e + y = p_{O_2}^c + p_{N_2}^c + p_{CO_2}^c + y \quad (8)$$

rearranging equation 8

$$p_{O_2}^c = p_{O_2}^e + p_{N_2}^e + p_{CO_2}^e - p_{N_2}^c - p_{CO_2}^c \quad (9)$$

Each of the right-hand terms in equation 9 can be expressed in known values by rearranging equations 4, 5, 6, 2, and 3 respectively in the manner shown below for equation 4

$$p'_{O_2} = \frac{p_{O_2}^e}{1 - \left(\frac{Ah \frac{273}{T}}{V_a^e \frac{273}{T} + \alpha_{O_2} V_f^e} \right)} \quad (10)$$

Having solved for p'_{O_2} by equation 9 in terms of initial values and known changes thereof, solution of equation 7 is now completed. In a similar manner the vessel conversion factor, F , is determined for CO_2 .

As there is no direct relation between x and h , values of Ah observed during the course of a typical experiment are used in solving for x . Oxygen exchange, x_{O_2} , is computed from the relation: $x_{O_2} = F_{O_2} \cdot h$ in the case of the one-vessel method, or x_{O_2} and x_{CO_2} are found from the two-vessel equations (45). For the volumeter with a capillary cross section of 0.202 mm.² the following factors were found:—

1. One-vessel method
 - (a) carbonate buffer $F_{O_2} = 0.212 \text{ mm.}^2$
2. Two-vessel method
 - (a) large liquid volume $F_{O_2} = 0.208$
 $F_{CO_2} = 0.277$
 - (b) small liquid volume $f_{O_2} = 0.216$
 $f_{CO_2} = 0.218$

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