as Bagley points out, an extended almost close-packed space-filling structure can be generated. It is a moot point whether or not such a structure could be considered a crystal.

GREGORY MILMAN
Harvard University,
Cambridge, Massachusetts
BETTY G. UZMAN, ANJA MITCHELL
ROBERT LANGRIDGE
Children's Cancer Research Foundation,
and *Harvard Medical School,*
Boston, Massachusetts

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Quantum Yield of Oxygen Evolution and the Emerson Enhancement Effect in Deuterated Chlorella

Abstract. *The maximum quantum yield of oxygen evolution in deuterated chlorella is found to be 0.075, while normal chlorella showed, in parallel experiments, a value of 0.10. Deuterated* Chlorella vulgaris *showed a decline in the quantum yield of oxygen evolution ("red drop") beginning at 680 millimicrons and a clear Emerson enhancement effect qualitatively similar to that obtained in normal chlorella cells. However, the ratio of quantum yield at 680 to that at 710 millimicrons was about 1.5 times higher in normal than in deuterated chlorella cells. Action spectra of the Emerson enhancement effect in deuterated chlorella also are qualitatively similar to those of normal cells.*

It is now widely accepted that photosynthesis requires two light reactions (*1*) and that, because of this, normal chlorella cells show the so-called Emerson enhancement phenomenon (*2*). The importance of deuterated organisms in biological research has been recently reviewed by Katz and Crespi (*3*). Whether the two light reactions are necessary for deuterated chlorella was uncertain, because Emerson (*4*) was unable to observe the enhancement effect in several samples of partially deuterated chlorella. We investigated the action spectrum of photosynthesis in completely (99.5 percent) deuterated *Chlorella vulgaris* and observed the usual decline in quantum yield of photosynthesis beyond 680 mμ ("red drop") and the Emerson enhancement effect.

Deuterated *Chlorella vulgaris* (obtained from J. J. Katz and H. L. Crespi) was cultured in inorganic salt medium (*5*), and the cells, harvested after 4 to 7 days' growth, were suspended in deuterated Warburg's buffer No. 9 (15 parts 0.1*M* K_2CO_3 and 85 parts 0.1*M* $NaHCO_3$ in D_2O) in a volume concentration of 0.4 to 0.5 percent. Measurements of O_2 evolution were made with a differential manometer (*6*). The manometric vessels, submerged in a constant-temperature bath (9.6° ± 0.1°C), were continuously shaken with a frequency of 200 oscillations per minute. Light (bandwith of beam, 5 to 10 mμ) from the large Emerson-Lewis monochromator (*7*) entered the reaction vessel from the bottom; supplementary (or background) light also entered the vessel from the bottom. Supplementary beams were produced by filtering white light with Baird-Atomic interference filters (60 to 80 percent transmission at their peaks) which had half-bandwidths of 8 mμ. Both light sources were tungsten lamps.

Quantum yield determinations required measurements of the number of absorbed quanta. The number of incident quanta was measured by a large-surface bolometer calibrated against a radiation standard from the U.S. Bureau of Standards. Percent absorption at different wavelengths was measured by two instruments: (i) a Bausch and Lomb (Spectronic 505) spectrophotometer, equipped with an integrating sphere and (ii) an integrating sphere spectrophotometer constructed by Cederstrand (*8*) in our laboratory. Aminco cuvettes were used in the Bausch and Lomb instrument, while in the other instrument manometer vessels were used directly. Measurements with the Cederstrand instrument were considered more reliable and were used for quantum yield calculations, but values obtained with the Bausch and Lomb instrument deviated from the former by less than 5 percent. All measurements (unless otherwise specified) were made in the linear part of photosynthesis versus light-intensity curve.

Table 1. Rates of O_2 evolution and O_2 uptake in deuterated and normal cells of *Chlorella vulgaris*. All results are expressed in millimicromoles per liter per hour.

Rate of O_2 evolution*		Rate of O_2 uptake†	
Deuterated cells	Normal cells	Deuterated cells	Normal cells
Weak white light (5%)			
496	625	315	289
Strong white light (100%; saturating)‡			
868	3022	360§	431§

* Rate of O_2 evolution was corrected for differences in percent absorption in the two samples. † Respiration rate (for same volume of cells) was calculated by taking the average of rate of O_2 uptake before and after illumination. ‡ Saturation of O_2 evolution checked by changing light intensity by placing 60-, 70-, and 80-percent transmitting neutral density filters and finding a constant rate of photosynthesis. § Respiration rate for chlorella was higher because the higher rate of photosynthesis with 100-percent light caused an increased O_2 uptake.

On the basis of a minimum of three experiments performed on different cultures for each of the phenomena reported, the following conclusions were reached for deuterated cells of *Chlorella vulgaris*.

In deuterated chlorella the rate of O_2 evolution measured at saturating light intensities was reduced by a factor of 3 (Table 1); when measured in weak light intensity the rate was reduced only slightly (about 20 percent). (Several other experiments gave qualitatively similar results.) The findings of Craig and Trelease (*9*) are thus partly confirmed—they had concluded that deuteration causes a decrease in the rate of rate-limiting chemical reactions but no difference in the rate of photochemical reactions. Rates of O_2 uptake were quite comparable in both deuterated and normal cells, except that after exposure to strong light normal chlorella showed a larger increase in rate of uptake than deuterated cells did (Table 1).

There is a definite decline ["red drop" (*7*)] in the quantum yield of O_2 evolution by deuterated cells at wavelengths beyond 680 mμ (Fig. 1). The maximum observed absolute quantum yield in deuterated chlorella was 0.075; chlorella grown in ordinary water under comparable conditions gave a maximum yield of 0.10. (We do not claim that higher values cannot be obtained under other conditions.) The

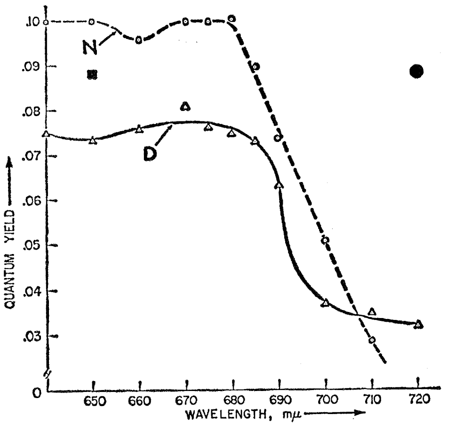

Fig. 1. Quantum yield of photosynthesis (O_2 evolution) as a function of wavelength of light for deuterated *Chlorella vulgaris* (curve D; triangles) and normal *Chlorella vulgaris* (curve N; circles). Note beginning of "red drop" around 680 to 685 mμ. Yield at 650 mμ in 714-mμ background light for deuterated chlorella is shown by a solid square, and yield at 720 mμ in 655-mμ background light by a solid circle. Temperature: 9.6°C.

ratio of quantum yield at 680 mμ to that at 710 mμ was 2.1 in the deuterated chlorella, against 3.3 in the normal chlorella. Deuterated cells show an almost constant yield in the range from 700 to 720 mμ. No such plateau could be established in H_2O-grown chlorella. Observations of a constant yield in the region between 700 and 720 mμ can be understood in the "parallel" formulation (*10*) of photosynthesis, if one assumes that both pigment systems (S_{II} and S_I) alone are capable of evolving O_2, but with widely different efficiencies. Perhaps deuteration has narrowed the gap between efficiencies of the two pigment systems, which enabled us to observe a constant yield of photosynthesis in the range from 700 to 720 mμ. An explanation in terms of the "series" model (*11*) seems difficult unless we assume that a large proportion of the long-wave form of chlorophyll *a* belongs to system II.

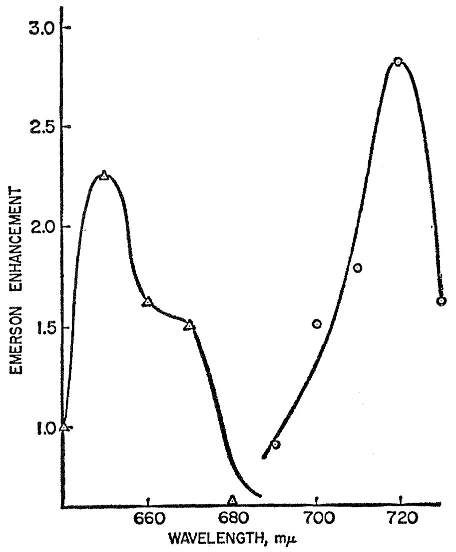

Fig. 2. Emerson enhancement as a function of wavelength of light (see text). Curve with triangles, far-red (714 mμ) background light; curve with open circles, red (655 mμ) background light. Organism: deuterated *Chlorella vulgaris*; temperature: 9.6°C.

In two out of three experiments with deuterated chlorella, the quantum yield of photosynthesis at 720 mμ in the presence of 655 mμ background light was higher than the maximum quantum yield of photosynthesis observed at any wavelength without background light. The separate-package model of photosynthesis [see Myers (*12*) and Bannister and Vrooman (*13*)] predicts such a result, but the spill-over model (*12, 13*) does not. However, we should look at this result with some reservation because of the low yield at 720 mμ (when used alone) and, therefore, the possibility of errors in measurements of yield in this light.

Deuterated cells definitely show the Emerson enhancement effect (Fig. 2). To insure maximum enhancement (see *14*), the intensity of the short-wave beam was so chosen that it alone produced five to seven times more photosynthesis than the long-wave beam alone. Enhancement was observed on the short-wave side of the region between 680 and 685 mμ when 714-mμ background light was used and on the long-wave side of the region between 680 and 685 mμ when 655 mμ background light was used. Enhancement (*E*) was calculated according to the following formula, which is based on the assumption that it is the low efficiency of far-red light which is enhanced.

$$E = R(S + L) - RS/RL,$$

where *R* stands for rate of O_2 evolution, *S* for short-wave light, and *L* for long-wave light. The action spectrum of the Emerson enhancement effect with 714-mμ background light shows peaks due to chlorophyll *b* at 650 mμ and a shoulder due to chlorophyll *a* at 670 mμ. This spectrum is only qualitatively similar to the one given earlier by Govindjee (*14, 15*) and by French and co-workers (*16*) for chlorella grown in ordinary water. Some differences may be due to deuteration, but we must recall that differences are also found between different cultures of chlorella (*14*). The action spectrum of the Emerson enhancement, made with 655-mμ background light, shows a peak around 720 mμ. The actual location of this peak may not necessarily coincide with absorption maximum of the long-wave form of chlorophyll *a* because (i) the action spectrum follows fractional rather than absolute absorption spectrum of the active pigment and (ii) the enhancement spectrum is a difference between spectra of systems I and II [French *et al.* (*16*) and Duysens (*17*)].

We have shown that deuterated chlorella cells are similar to normal cells as far as some of the basic photosynthetic processes are concerned, but some interesting differences are also present. Ghosh *et al.* (*18*) have recently shown that the fluorescence yield of deuterated chlorella is somewhat higher than those of normal cells; the shape and location of emission spectra are somewhat different in the two cases.

GLENN BEDELL
GOVINDJEE

Department of Botany, University of Illinois, Urbana

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Chemisorption of Water at High Temperatures on Kaolinite: Effect on Dehydroxylation

Abstract. *The dehydroxylation reaction of kaolinite in a vacuum at 425°C is halted by introducing a water vapor pressure of 47 mm-Hg, and is resumed when the vacuum is reestablished. The sample gains weight corresponding to an approximately monomolecular layer of water on the kaolinite surface. At the temperatures and vapor pressures involved, the sorption is considered to be a chemisorption process.*

For some years, evidence has accumulated that the dehydroxylation of the mineral kaolinite, $Al_2Si_2O_5(OH)_4$, which takes place at temperatures exceeding about 420°C, is markedly dependent on the ambient water vapor pressure, even at pressures of a few millimeters of mercury. This dependence is not due to any rehydroxylation of the mineral because this requires high water vapor pressures (*1*). When pressed discs of the mineral are heated at constant temperature under normal atmospheric conditions, the overall rate of dehydroxylation decreases progressively as the disc thickness increases (*2*); it appears that the water vapor generated by the reaction has a self-retarding effect. When a partially reacted disc is sectioned, the interior may be little reacted when the exterior layer is fully reacted, even though the entire sample has been held at the same temperature for the same time (*3*).

More precise information is obtained when samples are reacted slowly under controlled water vapor pressures (*4, 5, 6*). Results obtained by Toussaint *et al.* (*5*) and by Brindley *et al.* (*6*), the latter employing vapor pressures from less than 10^{-3} mm-Hg to 157 mm-Hg, suggest that the marked effect of the water vapor atmosphere arises from the sorption of water on the mineral surface, which effectively reduces the area for dehydroxylation. At the temperatures and vapor pressures involved, approximately 425° to 525°C and up to 150 mm-Hg pressure, the sorption must be regarded as a chemisorption process.

Experiments have been carried out which show directly the sorption of water on kaolinite under these conditions. The sorbed water is taken up rapidly and halts the dehydroxylation reaction, and when a vacuum is reestablished the sorbed water is released, and the dehydroxylation proceeds normally.

We used a silica spiral microbalance (*6*) in an enclosed system where the reaction temperature and the water vapor pressure were independently controlled. Reactions were followed gravimetrically on loosely compacted samples (~ 100 mg), and results are expressed as a percentage change in the weight of the sample dried at room temperature in a vacuum.

Typical curves are shown (Fig. 1) for samples maintained at 425° ± 2°C, partly in a vacuum at a pressure lower than 10^{-3} mm-Hg, and partly at a vapor pressure of 47 mm-Hg. Curve I shows a run in a vacuum for 210 minutes; at the end of this time the dehydroxylation reaction has become quite slow. Introducing the water vapor causes an almost instantaneous weight increase of about 0.85 percent; removal of the vapor restores the original weight. Curve II shows the same dehydroxylation process, but in vacuum for only 60 minutes at which time the reaction is still proceeding rapidly. Introducing the water vapor halts the reaction, and there is a weight increase of 1.30 percent, the weight remaining constant for 60 minutes while the vapor atmosphere is maintained. When the vacuum is restored, the reaction is resumed along a continuation indicated by the original curve, with a time displacement of about 80 minutes. Curve III shows a run in which the vapor was introduced immediately after the heated furnace was raised around the reaction vessel. Apart from a small initial reaction attributable to establishing steady conditions, there is no further dehydroxylation for 75 minutes, until the vacuum is applied, and then the reaction proceeds normally; curve III is practically parallel with curve I. Curve IV shows that when the water-vapor atmosphere is continued for 4 hours there is no reaction after the first small effect.

If the effective area of a sorbed H_2O molecule is taken as 10.8 Å^2, the weight of sorbed water shown by curves I and II, around 1.0 percent, corresponds to an area of about 36 m^2/g. The surface area of the initial kaolinite determined by the nitrogen adsorption method of Brunauer, Emmett, and Teller is 30 m^2/g. The correspondence of these areas points to the establishment of an approximately monomolecular layer of sorbed water on the kaolinite. Other experiments (*7*) indicate that the surface area of kaolinite is likely to be little changed by partial dehydroxylation at 425°C.

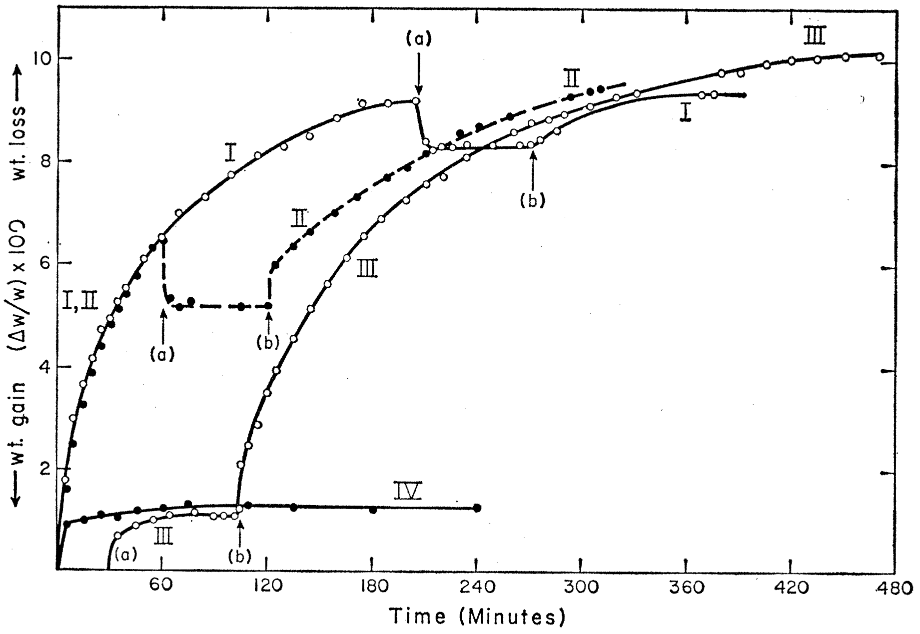

Fig. 1. Dehydroxylation of kaolinite at 425°C in a vacuum, or in a water-vapor atmosphere of 47 mm-Hg. Arrow (*a*) shows where water vapor is introduced, and (*b*) shows where the vacuum is restored.