Photosynthetic Units

GEORG H. SCHMID and HANS GAFFRON

From the Institute of Molecular Biophysics, Department of Biological Science, Florida State University, Tallahassee, Florida 32306. Dr. Schmid's present address is Max-Planck Institut für Züchtungsforschung, 5 Köln-Vogelsang, Germany

ABSTRACT Leaf tissues of aurea mutants of tobacco and *Lespedeza* have been shown to have higher photosynthetic capacity per molecule of chlorophyll, a higher saturation intensity, a simpler lamellar structure, and the same quantum yield as their dark green parents. Here we report on the values of photosynthetic units for both types of plants and some algae. The unit has been assumed to be about as uniform and steady in the plant world as the quantum efficiency. The number on which all theoretical discussions have been based so far is 2400 per O_2 evolved or CO_2 reduced. With dark green plants and algae our determinations of units by means of 40 μsec flashes superimposed on a steady rate of background photosynthesis at 900 ergs cm^{-2} sec^{-1} of red light yielded mostly numbers between 2000 and 2700. However, the photosynthetic unit turned out to be very variable, even in these objects. In aurea mutants the unit was distinctly smaller, averaging 600 chl/CO_2. By choosing the right combination of colors for flash and background light, units as low as 300 chl/CO_2 or 40 chl/e^- could be measured consistently. We found five well-defined groups of units composed of multiples of its smallest member. These new findings are discussed in terms of structural entities that double or divide under the influence of far-red light.

INTRODUCTION

The photosynthetic unit is experimentally defined as the number of oxygen molecules evolved or carbon dioxide molecules reduced per molecule of chlorophyll when the chloroplast pigments are excited by one flash of light so short that the set of enzymes involved in the process will not function twice during its lifetime, and so strong that a further increase in flash intensity does not change the measured value. By investing this formal expression with the meaning of a structural component in the photosynthetic apparatus, it is possible to reconcile three seemingly contradictory yet well-established observations. First, not one but at least eight quanta of light are necessary to accomplish the basic over-all photosynthetic reaction. Thus either the energy or the primary products of eight excitation processes must be collected. Second, in a plant exposed to dim light, photosynthesis proceeds with optimal efficiency

long before any single individual chlorophyll molecule in the light path has had the opportunity to collect eight quanta. Third, the same chlorophyll molecules which are so effective in a weak stream of light quanta, are unable to make full use of a very short intense flash of light which excites a great number of them "simultaneously"; i.e., within 10^{-5} or 10^{-6} sec. Because a saturating light flash causes only one carbon dioxide molecule to be reduced for each 2400 molecules of chlorophyll, thirty years ago 2400 molecules of chlorophyll were declared to constitute a unit which collects the energy needed for the multiquanta reduction process (7). After 8–10 quanta have been absorbed anywhere within a unit and their energy delivered to an enzymic "reduction center" the particular unit is out of commission for the next two hundredths or so of a second, the time it takes to complete the chemical transformations and to restore the initial sensitivity of the unit. This conception solved all three points mentioned above (16). The time needed to capture the number of quanta for the reduction of one equivalent of CO_2 per flash is cut by a factor of more than two thousand. Inasmuch as photosynthesis is an extended sequence of chemical reactions passing over presumably fairly stable intermediates—so it was soon pointed out (26)—it is permissible to scale down the size of the unit to serve the formation of a sufficiently stable intermediate. For instance, the appearance of each one of four reducing hydrogens requires the cooperation of 600 molecules of chlorophyll; or the effective transfer of one electron by an excitation process within a cluster of 300 pigment molecules. Thirty years of discussion of this problem reflected in several comprehensive reviews (33, 36) have made it clear that no theoretical objections exist against the assumption of a mechanism for photon energy transfer within such a unit, particularly within the smaller "partial" units. Nevertheless we have recently been reminded of the reality of some kind of big unit, which conforms to Emerson and Arnold's original flash saturation number, by the experiments of Izawa and Good, who showed that one molecule of a poison which specifically prevents the release of oxygen suffices to inhibit the concerted action of 2400 molecules of chlorophyll (21).

While the picture of a great number of essentially equal light-harvesting pigment molecules in combination with one reaction center was deemed sufficient for many years, the more recent evidence for the existence of two or more coupled photochemical reactions, each with their own pigments attached to their own electron transport chains, requires a much more sophisticated approach to the unit problem, particularly because there has been also a tendency to identify granular constituents of chloroplast lamellae as seen in electron photomicrographs with the unit or its parts.

In recent publications we have compared the structural appearance of chloroplasts from normal green and yellow tobacco leaves with the type and the kinetics of their photosynthetic reactions (19, 38, 40, 42). The results have

encouraged us to take up the question of whether photosynthetic units are as stable and unchangeable as, for instance, the optimal quantum efficiency, or whether units might shift in size with chlorophyll content and saturation rates. Preliminary results presented two years ago (39) showed that we could expect a number of complications due to known induction phenomena (5, 9, 13). Our new results demonstrate that photosynthetic units (as defined above) vary considerably between 300 and 5000 chl/CO_2, though the value encountered most often with a normal green leaf picked at random or the usual culture of algae is indeed the classical 2400 chl/CO_2.

We first expected the variations to be of a purely statistical nature, a continuous drift between all possible values in the range mentioned. But after a great number of determinations we are now sure that the changes take place in well-defined steps, which can be arranged in the following series of values n for n chl/CO_2: $\backsim$300; $\backsim$600; $\backsim$1200; $\backsim$2400; $\backsim$5000.

It is inevitable that this statement will set in motion a great deal of speculative "model building", all of which would be for nothing if the supporting measurements themselves were not reliable. In this paper the accent is therefore on the description of the method which permitted us to come to such a conclusion, and of the conditions which evoke one unit size in preference to another.

MATERIALS AND METHODS

I. *Plant Material*

(*a*) REQUIREMENT FOR BASIC UNIFORMITY

The plant material we used falls into two categories: first traditional, such as cultures of *Chlorella* and other algae with well-known and quasi-standardized properties; second unusual, such as cut leaf sections from different strains of tobacco and other higher plants with new and variable traits. While it is fairly easy to obtain sufficiently uniform algal cultures to run all the tests desired, it takes patience and experience to grow plants with leaves which can be relied upon to give reproducible results. Not only must leaf sections for double or triple tests be alike in their average properties, but also the majority of the very cells of which the tissue is composed ought to be in the same stage of development and respond in the same manner and to the same extent to conditions imposed from outside. Such uniformity is not crucial for determinations of the optimal quantum efficiency because the latter remains the same regardless of how much such leaf sections may vary in terms of the age or the size of their grana and their chlorophyll content (41). By contrast the units vary with age, pigment content, and the pretreatment of the leaf tissue. It would be impossible to give a proof for a stepwise variation of units by working with a leaf tissue which consists of a fine mosaic of cells or clumps of cells with widely different chlorophyll contents and sizes of units. It becomes important, therefore, to watch for the slightest damage to the plants by aphids or virus, or careless spraying of the leaves with drugs to counteract

such infections. Such events are likely to produce a mottled state due to dissimilar conditions of neighboring individual cells. All such leaves must be discarded. On the other hand, very large patches of dissimilar cells within one leaf, such as appear in variegated plants, are good objects to study provided all cells in a lighter or darker patch are sufficiently alike (19, 37).

In order to be able to compare leaf tissue and unicellular algae the algae were deposited on a wet surface by filtering through a Millipore filter and then treated in the same way as the leaves.

(*b*) HIGHER PLANTS

Most experiments were made with leaf cuts from the Connecticut cigar variety, John Williams Broadleaf, of *Nicotiana tabacum* L. and its aurea mutant Su/su which emerged from the same seed population. All these plants were grown in an air-conditioned greenhouse in 1967. The data of Fig. 6 *c* were obtained with *N. tabacum* L. aurea from "Japanese Bright Yellow," a tobacco with so-called "White Burley" character, which will be described in detail elsewhere. Adult plants of the latter variety lose much of their chlorophyll in the lower leaves and stem. If the usual commercial practice is followed and the plants are topped by breaking off the stem several nodes below the seed head, the loss of chlorophyll in this plant, as in all tobaccos with White Burley character, is increased. Within a short time the plants become very light colored. Except for a slight mottling of the leaves as they ripen, dark green varieties retain their color after this treatment. The developmental state of the plants at the time their leaves were picked is best characterized by their chlorophyll content as indicated in the legend of Fig. 6.

Plants of *Cassia obtusifolia* were either collected outside or planted in flower pots and grown in the same greenhouse.

(*c*) ALGAE

The algae, *Anacystis nidulans* (Richter) and *Scenedesmus obliquus* D_3, were grown as usual and synchronized in a 10 hr dark/14 hr light cycle. Manganese-deficient *Ankistrodesmus braunii* 202-7c were kindly grown for us by Dr. E. Kessler (25). The manganese deficiency was produced in media with "Specpure" (Johnson Matthey Chemicals Inc., London, England) chemicals in especially deionized water. Vanadium-deficient *Scenedesmus* D_3 were grown for us by Dr. P. Homann.

II. *Flashing Devices*

The Multiflash model 553 from E. G. and G. Inc. (Boston, Mass.) has a xenon light source. At high output the average flash energy is approximately 12 w·sec. The peak intensity is 2 million lux at 1 meter distance at the center of the beam. The flash duration is 40 μsec at one third of the peak intensity. The time from switching the device to complete darkness is 100 μsec. The device can be triggered for single bursts or a series of bursts and at two different intensities. The flash energy can be computed from the number of bursts in a 2 min period (cold start), the flash rate (flashes/sec), and the burst duration (in sec). According to these data the flash energy may reach 26 J. The reflector gain is approximately 10.

Energy Intercepted by a Leaf Section The unit beam candlepower provided by the manufacturer gives little information about the energy at the light source. The photometric units are tied to the response of the human eye. Since the spectral distribution of xenon flash lamps is well-known, the photometric unit can be used to determine approximately the actual spectral energy content of the flash lamp. The radiance of a 2×10^6 candlepower beam is equivalent to 1.39×10^{-6} w/lumen · A $\times$ 2×10^6 lumen/steradian = 2.78 w/lumen · A at 5600 A. In the paper by Gonz and Newell the spectral distribution of a pulsed xenon source is given (17). It can be seen that the spectrum extends over about 1000 A with the main wavelength at 5600 A. The total radiometric intensity for the flash then is approximately 2780 w/steradian. A leaf section of 3 cm^2 at 25 cm distance subtends a solid angle of $3/(25)^2$ steradian or 4.8×10^{-3} steradian. The power intercepted by the leaf section is 2.78×4.8 w = 13.4 w/flash. The energy intercepted by a 3 cm^2 target is approximately 1.34×10^{-3} J $= 1.34 \times 10^4$ ergs per xenon flash. Light intensities of the flashes were lowered by putting wire screens in the light path. The effect of the screens was measured in continuous light with a xenon lamp (Osram XBO, 150 w).

The model 549-11 flash unit from E. G. and G. gives very brief flashes. The flash unit is an air flashtube (guided spark-gap light source). Total time from switching to darkness is 3 μsec. At one third peak intensity the flash duration is only 5×10^{-7} sec. The peak light intensity is 50×10^6 beam candlepower in terms of the manufacturer. A beam candle is comparable to the same value of lux at 1 meter distance at the center of the beam. 50×10^6 beam candles are 50 million lux under the above conditions.

This flashing device can only be triggered every 5 sec. The light spectrum extends from 0.36 to 0.64 micron. This means over 2800 A. At 25 cm a 3 cm^2 leaf section intercepts the power of 940 w/flash. The energy intercepted by a 3 cm^2 target is approximately $940 \times 3 \times 10^{-6}$ J $= 2.8 \times 10^4$ ergs per air flash.

Number of Flashes per Burst Time The Multiflash 553 does not necessarily give the number of flashes per burst time that the manufacturer's manual indicates. We therefore counted the actual number of flashes by recording the discharge noise of the flashtube on a tape recorder at high speed and playing it back at low speed. Otherwise the machine worked perfectly and every detail once checked remained very reproducible.

Comparison of Our Flashing Devices with Those Described by Allen and Franck and the One Used by Emerson and Arnold The flashing device of Allen and Franck (1) differed in several respects from ours. They had an energy input of maximal 800 w·sec (100 μF and 8 kv) with a peak intensity of 2.5 million lux. Our xenon flash had a power input of 12 w·sec (5 μF, 2.2 kv) and a peak intensity of 2 million lux. At one-half of the maximal intensity they had an intensity of 7000 lumen sec compared to 7500 lumen sec under our conditions. Our airflash had an input of 6.5 w·sec (0.5 μF, 16 kv). The main difference between their device and ours lies in the flash duration. Their time at half-width was 0.5×10^{-3} sec long, ours, 4×10^{-5} sec, that is one order of magnitude shorter. Our airflash tube gave still shorter flashes with a time at half-width of 5×10^{-7} sec.

The flashing device of Emerson and Arnold (7) was a neon tube with a power input of only 4.5 w·sec (1 μF, 3 kv). The mirror gain was 3 to 4. All our flashes gave more light than theirs. They used either 12 or 21 bursts/sec uninterruptedly for 5 min at a time.

Checking Flash Saturation and Recovery Times The first prerequisite for a valid flash experiment is that the intensity of the flash really saturates the photosynthetic mechanism. Fig. 1 is a plot of carbon dioxide fixed per flash against increasing flash intensity. The curves show that the quantum flux was more than sufficient to saturate the flash fixation capacity of our plants. Independently of the actual amount of CO_2 fixed the curves can be used for a rough estimate of the ratio between the number of chlorophyll molecules present and of quanta absorbed when saturation sets in. Take for instance *Cassia* and Su/su in this figure. In *Cassia* a leaf target with 175 γ total chlorophyll/2.25 cm^2 (10^{17} chlorophyll molecules) became flash saturated when about 20% of the energy of 1.34×10^4 ergs/flash, or approximately 5×10^{14} quanta, were intercepted by the leaf (44). This amounted to 200 chlorophyll molecules per absorbed quantum or a "unit" of an order of magnitude of 200 per electron transfer.

On the other hand the CO_2 increment for *Cassia* in Fig. 1 yields a CO_2 unit of about 1000. The same calculation for a Su/su leaf with 19.5 γ total chlorophyll/3 cm^2 showed that 1.18×10^{16} chlorophylls absorbed 7×10^{14} quanta when flash saturation had been reached which would correspond roughly to 20 chl per e^-. The CO_2 increment of the Su/su curve gave 300 chl per CO_2 fixed. Fig. 1 makes it clear that more light was needed to saturate the small unit than the large one. It follows that the input flash energy of 4.5 w·sec of Emerson and Arnold would not have sufficed to saturate the unit of our aurea mutant, and thus these authors would not have been able to discover the existence in their algae of any unit much smaller than what they found.

The second prerequisite for successful flash experiments is to have the right dark times between flashes. Fig. 2 sums up experiments which showed that at 18°C the flash yield decreased to half the saturation value when the dark intervals were shortened to 0.016 sec. This result agrees well enough with the value of 0.01 sec at 25°C given in the literature (7). If the dark interval relates to the turnover time of an enzyme system, it is not too surprising that in this respect yellow and green tobacco behaved alike.

Background Light Ever since Franck and Allen (1) found that working in a dimly lit room or a completely darkened one made a difference in the metabolic response to single strong flashes, it was clear that the disturbances known as induction periods since Osterhout's paper in 1918 (32) have to be taken into account very carefully also in flash experiments.

The intensity of our background illumination was measured with a factory-checked ISCO Spectroradiometer (Instrument Specialties Co., Lincoln, Neb.); the light actually absorbed by the leaf was measured with a large surface bolometer (41).

Wide band red or blue background illumination was obtained with either a 150 w cool beam spotlight (General Electric) or a Sylvania "sungun" lamp and appropriate filters (41). 5 min before and during the flashing period we illuminated with 900

ergs cm^{-2} sec^{-1} of red light $580 < \lambda < 700$ mμ; or 540 ergs cm^{-2} sec^{-1} of blue light $380 < \lambda < 575$ mμ; or 6600 ergs cm^{-2} sec^{-1} of far-red light $700 < \lambda < 750$ mμ. A normal green leaf section absorbed approximately 80% of the red, 100% of the blue, and 5–10% of the far-red light. This regimen with $^{14}CO_2$ in air was preceded by 10 min of photosynthesis in ordinary air/$^{12}CO_2$ and in somewhat stronger light, namely 2400 ergs cm^{-2} sec^{-1} of red, 1000 ergs cm^{-2} sec^{-1} of blue, or 6600 ergs cm^{-2} sec^{-1} of far-red. In this manner we were able to avoid a large part of various induction troubles. The right duration of the preillumination treatment appeared to be more important than the intensity, and far-red had the best effect.

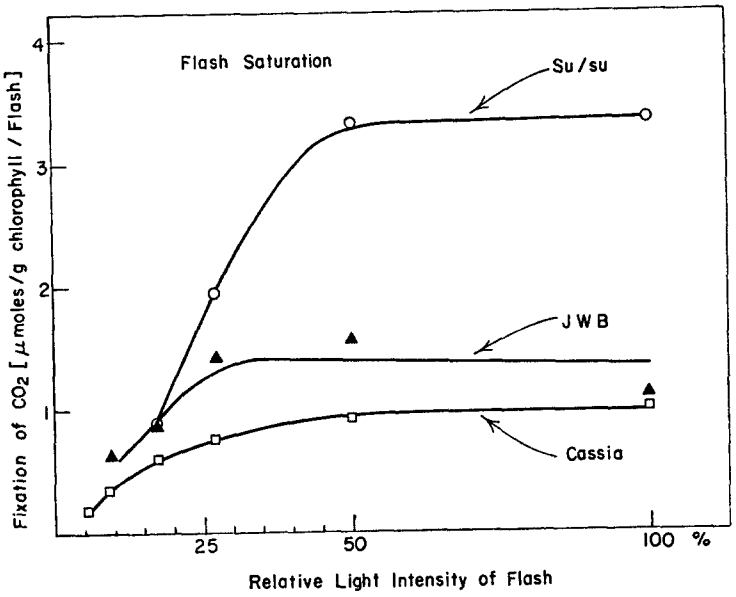


FIGURE 1. White xenon flashes on 900 ergs cm^{-2} sec^{-1} of red background light. Temperature 18°C.

III. *Measuring $^{14}CO_2$ Fixation—Alternating Use of ^{12}C and ^{14}C*

The classical experiments which led to the discovery of flash saturation and the photosynthetic unit were manometric gas exchange measurements, mostly presented as microliters of O_2 evolved. All our basic experimental data were obtained as fixation or loss of radioactive $^{14}CO_2$. No attempts have been made to ascertain how much the distribution of fixation products induced by a flash differs from that which prevails in continuous illumination. We also do not know whether the assimilatory quotient, the ratio $+\Delta\ O_2/-\Delta\ CO_2$, deviates from unity even in those flash experiments in which induction phenomena, which figure so prominently in single flashes at the beginning of a series, have been eliminated by various procedures. Nor do we know yet whether units for oxygen evolution vary exactly in the same manner as those determined for $^{14}CO_2$ fixation. It would be an interesting surprise if differences could be discovered.

For reasons that will be discussed further on, values for flash saturation relate to a unit only when the functions of different parts of the photochemical apparatus are in that dynamic equilibrium which corresponds to a steady quite measurable rate of photosynthesis. In most cases, therefore, the flashes were superimposed on a background illumination. A correction determined by counting the $^{14}CO_2$ fixed in a non-flashed control is easy to apply, if this background is put on only a short time before the flash experiment begins. But a short preillumination with the background light rarely achieves what it is supposed to do—namely establish a steady state of photo-

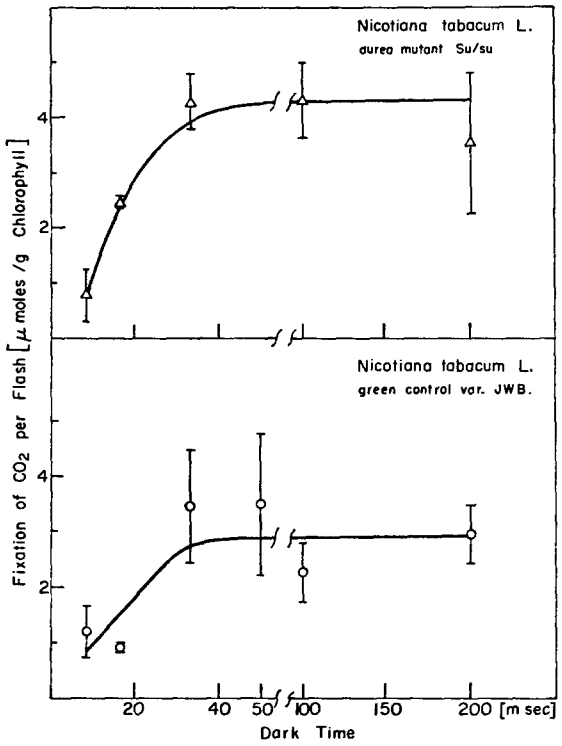


FIGURE 2. White xenon flashes on 900 ergs cm^{-2} sec^{-1} of red background light.

synthesis. This may take many minutes and during that time the amount of $^{14}CO_2$ fixed to be subtracted as a correction becomes so large as to destroy the required accuracy of the determination of the little extra $^{14}CO_2$ fixed by the superimposed flashes.

This problem was solved by the device explained in Fig. 3. The principle consists of alternating between two equal flows of a carbon dioxide–containing gas, one with ordinary $^{12}CO_2$ and the other with radioactive $^{14}CO_2$. It takes only the time to turn two stopcocks and to sweep out the reaction vessel, plus the time it takes for the new gas to diffuse into the leaves, to shift from a carbon dioxide assimilation which "counts" to one that does not. In this way all times during which $^{14}CO_2$ is being fixed, whether dark, or with background light only, or both superimposed with flashes, can

be standardized, while the pretreatment in $^{12}CO_2$ may be designed in any way one wishes for any length of time. What happens during the pretreatment can, of course, be determined separately—but does not necessarily enter into the calculation of the flash effects.

The carbon dioxide concentration was chosen so as to neither narcotize nor to deplete the photosynthetic mechanism. The influence of stomata movements in green and aurea leaves could be discounted (40, 41, 48).

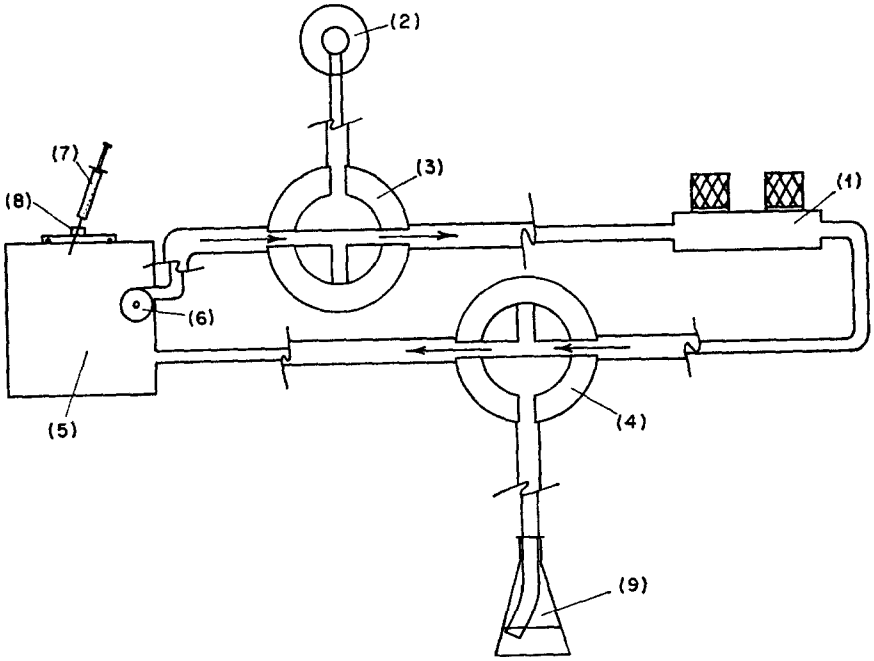


FIGURE 3. Diagram of the apparatus. (*1*) reaction vessel 6.08 ml with two wide stoppers to insert samples; (*2*) gas tank; (*3*) and (*4*) three way stopcocks; (*5*) Plexiglass gas chamber, 1857 ml; (*6*) miniature squirrel cage blower; (*7*) syringe; (*8*) surgical cap; (*9*) Erlenmeyer flask with saturated $Ba(OH)_2$.

In the schematic drawing of Fig. 3 the reaction vessel (*1*) had a total volume of 6.08 ml. It contained two thin metal wires on which the leaf sections (3 cm^2 for the yellow plants and 2.25 cm^2 for the green plants) were placed upside down. All illumination came from below. The vessel was first flushed for 15 min with a water-saturated mixture of 0.5% CO_2 in air (*2*).

During this equilibration procedure the vessel was illuminated for 10 min with high background light and for 5 min with low, as described in the preceding section. After a total of 15 min the valves (*3* and *4*) were turned to the position shown in Fig. 3, whereupon the radioactive gas contained in the Plexiglass chamber (*5*) began to circulate through the reaction vessel impelled by a small squirrel cage blower (*6*). The gas (volume 1857 ml) contained, besides 0.5% CO_2 in air, approximately 0.5 mc of radioactivity as $^{14}CO_2$. The mixture was made up by decomposing 70–73 mg of

cold $BaCO_3$ reagent grade and 2–5 mg of $Ba^{14}CO_3$ (Calbiochem, Los Angeles, Calif.), with 2 ml of 10% H_3PO_4 injected with a syringe (*7*) through a surgical cap (*8*) on the lid of the gas chamber. The temperature of the gas was kept at 18°C. The flashing started 30 sec after switching from $^{12}CO_2$ to $^{14}CO_2$. In most cases there were 25 sets of 14 flashes each. Each set of 14 flashes lasted 1.5 sec. The sets were spaced 5 sec apart (35, 39). Precisely 2 min and 30 sec later the flashing was stopped. Another 30 sec later, that is precisely 3 min after the labeled CO_2 had been admitted, the background illumination was switched off and the reaction vessel flushed with cold 0.5% CO_2 in air. The radioactive gas left in the reaction vessel (ca. 6 ml) was swept out and trapped in a saturated $Ba(OH)_2$ solution (*9*). 1 min later the leaf sections were taken out, killed by dipping into a very dilute solution of "flexible" collodion (Merck & Co., Inc., Rahway, N. J.), and glued to aluminum planchets. (Other ways of killing gave the same results.) The proper correction of counts lost by absorption in the collodion skin was determined by treating a test planchet of 0.12 μc ^{14}C (New England Nuclear Corp., Boston, Mass.) in the same manner. After 2 days of drying at room temperature, the leaf samples were counted in an automatic planchet counter (Nuclear-Chicago Corp., Des Plaines, Ill. Model No. C-110B). The absorption of counts inside the leaf itself was determined empirically by gluing increasing stacks of dried leaf sections on the same test planchet, and plotting the exponential decrease of the measured radiation. The radioactivity of the gas in the chamber was checked between experiments by taking gas samples with a 5 cc syringe (*7*) through a surgical cap (*8*). These samples were bubbled slowly through a freshly prepared and filtered $Ba(OH)_2$ solution. After adding 0.1 ml of 0.1 molar $NaHCO_3$ the precipitate was allowed to sit for several hours in a closed test tube, filtered through a 3 μ Millipore filter, and washed with distilled water. The filter was glued to the aluminum planchet and dried overnight at room temperature. The addition of cold CO_2 as $NaHCO_3$ makes $^{14}CO_2$ losses during the drying time insignificant.

The concentration of total CO_2 in the reservoir of radioactive gas was checked regularly by taking 10 ml gas in the manner described and converting it to $BaCO_3$. The precipitate was filtered, washed, and the carbon dioxide measured manometrically by decomposing the $BaCO_3$ with H_3PO_4 in a Warburg vessel. Empty filters were treated with $Ba(OH)_2$ and used as controls. Our commercial CO_2 gas mixtures were analyzed in the same way.

Carefully cut green and yellow leaf sections were used together in the same vessel at the same time; thus we were sure that our results for green and yellow tobacco remained comparable in every respect. This means, for example, that the striking differences in Figs. 1, 2, 4, and 5, etc. between yellow and green tobacco were established in the same vessel at the same time.

With 6–8 wk old plants we used one half of a small leaf for the flash experiment and the other half for the dim light control. The immediately adjoining leaf area was used to determine the chlorophyll concentration (38). From time to time, particularly when changing the flash colors, it was necessary to check whether our flashes were still saturating. Furthermore, some leaves were sensitive to too much light. The background fixation rate became inhibited at the normal flash intensity. A full flash gave a lower flash yield than a flash half as strong (1, 30). This effect was hard

to reproduce at will and depended on the state of the plant material. On the other hand, full intensity was needed to saturate especially the yellow leaf sections. A single determination of a flash yield often required three controls instead of the usual two.

Algae taken out of the culture medium were collected on a wet 3 μ Millipore filter. They formed a very evenly distributed layer of cells. After the experiment the algae were killed by dipping the filter into liquid nitrogen.

Considering the meaning of the results so obtained we must point out that anything which can possibly go wrong with the method tends to come out as too high a flash fixation of CO_2; i.e., as a unit that is too small. Therefore the method has to be applied with the utmost precision. Incomplete chlorophyll extraction and an incorrect determination of the standard counts are the most sensitive parts of the procedure. A gas leak in the device makes the specific activity determination wrong in the sense that the unit also comes out too small. We are aware that since we do not give relative units for light intensities, light absorptions, or flash increments but absolute numbers, our results may in the future be more easily criticized than if we had shown only relative units which would make a direct check impossible. We believe, however, that the averages of our unit sizes are too small by not more than 15%. The point is that it does not really matter whether the basic unit measures 300 or 350.

The situation is exactly the same with our method as in Izawa and Good's paper (21). If the number of inhibition sites per chlorophyll comes out wrong it gives a too small unit. The situation is also reminiscent of difficulties described by Homann (18) that what might go wrong when extracting manganese from chloroplasts is that one might find too much manganese per chlorophyll; e.g., the important manganese is only a fraction of the total manganese content.

RESULTS

Photosynthetic Units for CO_2 Fixation

The results are divided into three sections. First, results obtained with white (xenon) flashes on red background, as described in Methods. These constitute the bulk of our experiments. Second, results obtained with color combinations acting on carefully selected comparable material at the same time (within a few hours). Third, induction phenomena such as flash-induced losses of carbon dioxide, particularly in tissues incapable of complete photosynthesis.

1. WHITE XENON FLASHES ON RED BACKGROUND

The basic new fact which the present paper establishes is that the flash saturation value (see Methods) called "photosynthetic unit" (U) may vary in discrete steps from 300 to 2400 and beyond. The distribution of units according to size and numbers of observations has been given in graphs. (The ordinates indicate the per cent of experiments, the abscissae the sizes of U in terms of molecules of chlorophyll per molecule of CO_2 fixed per flash.)

In the course of the first experiments we gained the impression that the U values fell into certain groups. We therefore divided the entire range of values

into these groups. With a greater number of experiments the standard deviation became very small for the grouped averages, which indicated their high statistical significance. The groups arranged themselves into multiples of a U value of ∽300 as all our Figs. 4 to 11 show. Within each group the values show a perfect Gaussian distribution.

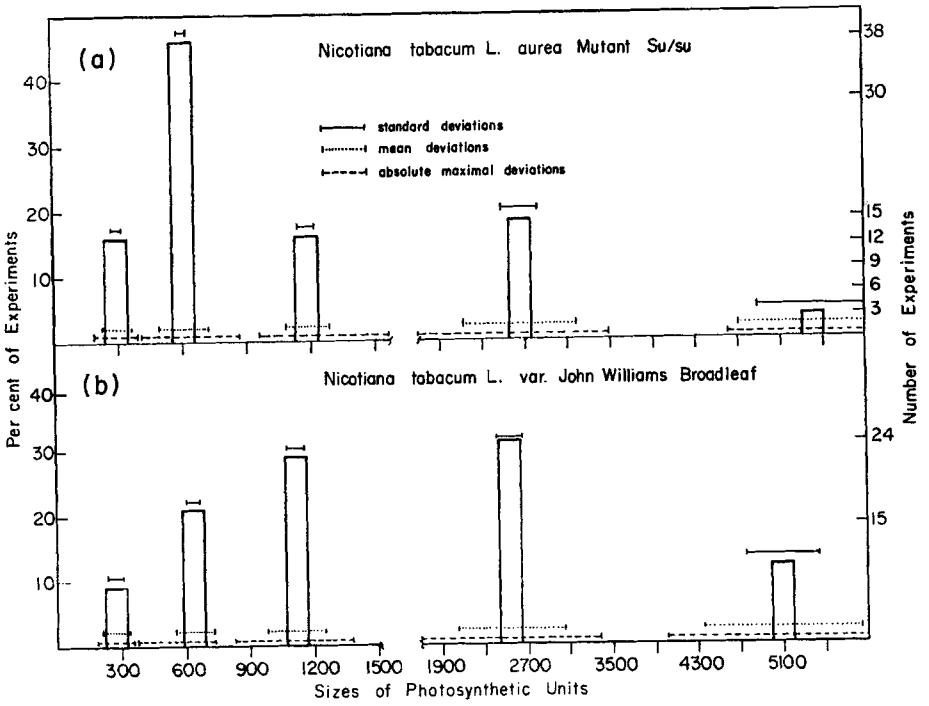


FIGURE 4. (*a*) *Nicotiana tabacum* L. aurea mutant Su/su, young plants 6–8 wk old. 82 flashed samples, 147 controls. Leaf area for the assay 3 cm². 229 determinations of chlorophyll in 3 cm². Highest total chlorophyll content 13 γ/cm²; lowest total chlorophyll content 1.2 γ/cm²; average 5.9 γ/cm²; standard deviation ± 0.14 γ/cm². (*b*) *Nicotiana tabacum* L. var. John Williams Broadleaf, young plants 6–8 wk old. 77 flashed samples, 143 controls. Leaf area for the assay 2.25 cm². 220 determinations of chlorophyll in 1.5 cm². Highest total chlorophyll content 76.3 γ/cm²; lowest total chlorophyll content 8.6 γ/cm²; average 22.1 γ/cm²; standard deviation ± 0.563 γ/cm².

Because the units increase roughly with the power of two, the scales of the abscissae have been shortened by cuts where virtually no units were found and by condensing the scale in the direction of the larger units. Any investigator who would have done just half a dozen determinations with the normal green tobacco John Williams Broadleaf (Figs. 4 *b*, 5 *b*), or *Cassia obtusifolia* (Fig. 6 *b*), or the algae *Anacystis nidulans* (Fig. 7 *a*) and *Scenedesmus obliquus* D_3 (Fig. 7 *b*), would have to come to the conclusion that his results had merely confirmed the classical data of Emerson and Arnold, for the spread of single values in the

series of experiments just mentioned is such that it seems to cover evenly the entire range shown. Note the spread of maximal deviations which did occur at least once in each group. Thus a few determinations could easily give the impression that carbon dioxide fixation units fluctuate between 300 and 5000 in the manner of a Gaussian distribution curve having a maximum around 2500. Such a result could either be blamed on the method, or explained

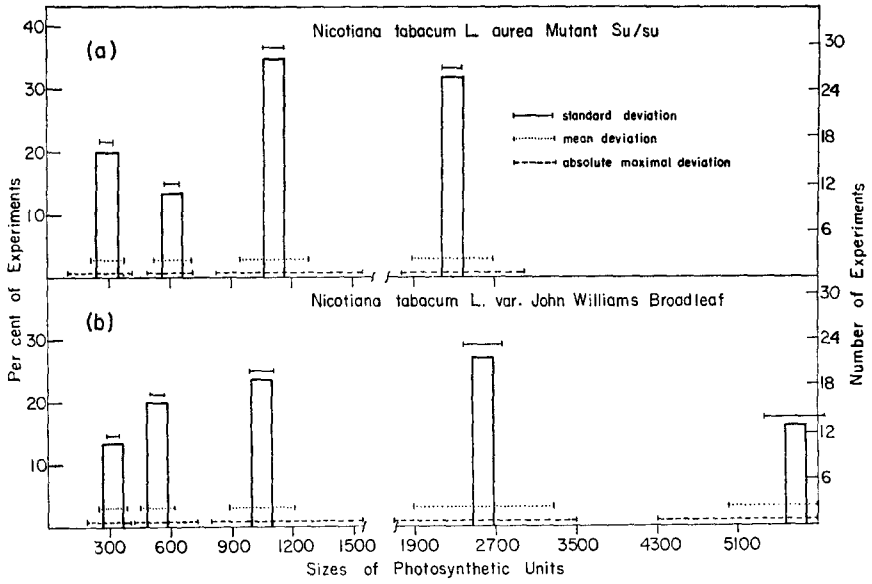


FIGURE 5. *Nicotiana tabacum* L. aurea mutant Su/su, growing leaves from adult plants. 60 flashed samples, 61 controls, leaf area for the assay 3 cm^2. 121 determinations of chlorophyll. Highest total chlorophyll content in 3 cm^2 17.9 γ/cm^2; lowest total chlorophyll content 3.1 γ/cm^2; average total chlorophyll content 9.7 γ/cm^2; standard deviation $\pm$ 0.27 γ/cm^2. (*b*) *Nicotiana tabacum* L. var. John Williams Broadleaf, growing leaves from adult plants. 60 flashed samples, 57 controls, leaf area for the assay 2.25 cm^2. 117 determinations of chlorophyll. Highest total chlorophyll content in 1.5 cm^2 64.5 γ/cm^2; lowest total chlorophyll content 13.8 γ/cm^2; average total chlorophyll content 41.3 γ/cm^2. Standard deviation $\pm$ 0.86 γ/cm^2.

physiologically as an imperfect reflection of a stable and rigid unit for the evolution of oxygen in a loosely coupled mechanism for carbon dioxide fixation.

Yet the simultaneous set of determinations with the tobacco aurea mutant Su/su (Figs. 4 *a* and 5 *a*), tobacco aurea from Japanese Bright Yellow (Fig. 6 *c*), and Swiss chard (Fig. 6 *a*), showed a very different and asymmetrical distribution of data. Obviously the method itself was not to blame. The reasons for the variation in size of units were, therefore, physiological ones and the simple expedient of repeating our measurements often enough proved

that the variations were not of a statistical nature, but occurred in distinct groups.

A comparison of all data we have, has forced on us the idea that there appear to be "forbidden" numbers. Between the separate groups the spaces are un-

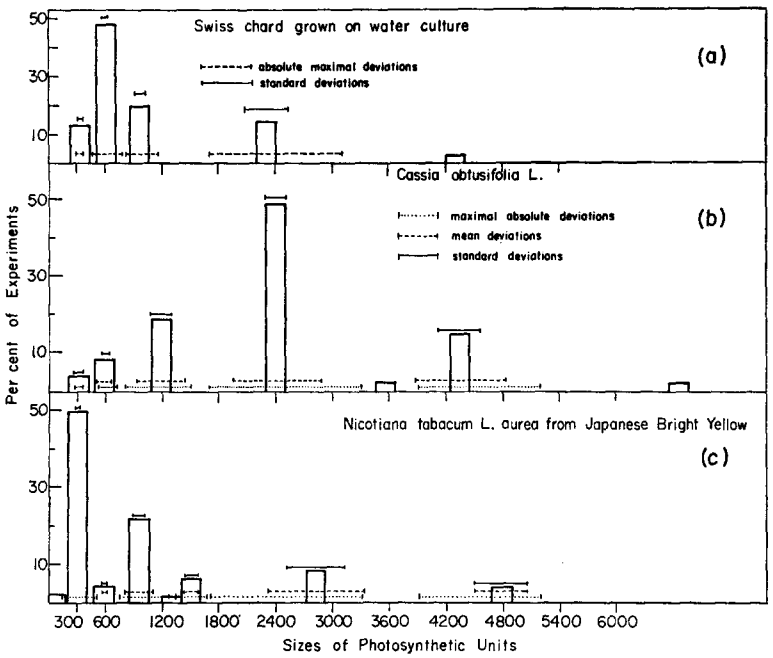


FIGURE 6. (*a*) Swiss chard. Growing leaves of water culture plants. 35 flashed samples, 31 controls, leaf area for the assay 2.25 cm^2. 66 determinations of chlorophyll in 1.5 cm^2. Highest value 71.6 γ/cm^2; lowest value 27.1 γ/cm^2; average value 43.8 γ/cm^2; standard deviation $\pm$ 1.245 γ/cm^2. (*b*) *Cassia obtusifolia* L. 47 flashed samples, 52 controls, leaf area for the assay 2.25 cm^2. 98 determinations of chlorophyll in 1.5 cm^2. Highest total chlorophyll content 78.4 γ/cm^2; lowest total chlorophyll content 37.5 γ/cm^2; average 58 γ/cm^2; standard deviation $\pm$ 1 γ/cm^2. (*c*) *Nicotiana tabacum* L. aurea from Japanese Bright Yellow. 46 flashed samples, 55 controls. leaf area for the assay 3 cm^2. 101 determinations of chlorophyll in 3 cm^2. Highest total chlorophyll content 8.5 γ/cm^2; lowest total chlorophyll content 0.7 γ/cm^2; average value 3.6 γ/cm^2; standard deviation $\pm$ 0.19 γ/cm^2.

expectedly clean, though the absolute unit value for corresponding groups of units (multiples of about 300) varies a little from plant to plant or for the same plant from one period of growth to another. Much larger are the variations for the probability of finding a particular size of unit to be the dominant, characteristic one for the plant sample in question.

Many hundreds of experiments with white flashes on red background made it clear that units vary stepwise according to certain rules, but did not provide us with a single self-evident clue why they varied in this manner. The reader

will easily find a correlation between larger units—particularly the classical unit of 2400—with higher chlorophyll content and increasing age of the plant (Fig. 5). But exceptions to such a rule are too clear to be ignored. See the comparison between Swiss chard with 44 γ/cm^2 chlorophyll and tobacco var. aurea from Japanese Bright Yellow in Fig. 6 with 3.68 γ/cm^2 chlorophyll. We also failed to find a convincing trend during the life cycle of synchronized algae (Table I). The experiments are sufficiently difficult that we do not want to attach much significance to the difference between 1200 at noon and 1900

TABLE I

PHOTOSYNTHETIC UNITS IN THE LIFE CYCLE OF SYNCHRONOUS *Scenedesmus* D_3

Stage in life cycle	Actual time	No. of experiments	Fixation of CO_2	Photosynthetic unit	Description of the algae
			μmoles/g chl/flash	*molecules chlorophyll/molecule CO_2 fixed/flash*	
After					
10 hr dark	9 a.m.	8	0.67±0.15	1500	Small cells which had just divided
2 hr light	11 a.m.	1	0.718	1390	Small cells growing
4 hr light	1 p.m.	8	0.85±0.146	1170	Growing cells distinctly bigger than at 9 a.m.
6 hr light	3 p.m.	4	0.615±0.53	1640	Growing cells
10 hr light	7:30 p.m.	4	0.535±0.205	1870	Big cells full of starch

Life cycle: 10 hr dark. 14 hr light.

Background light: red plastic filter $575 < \lambda < 700$ mμ spotlight. Preillumination during 10 min 2400 ergs cm^{-2} sec^{-1} and then 5 min 900 ergs cm^{-2} sec^{-1}.

Flashing: during flashing period 3 min 900 ergs cm^{-2} sec^{-1}. Flashes 14 in 1.5 sec 5 sec apart 25 times. Pulse duration at one third peak is 40 μsec. Total duration from light to darkness after switching the device is approximately 100 μsec. Xenon flash 553 Multiflash model from E. G. and G.

in the evening. This unsatisfactory situation fortunately changed partly when we proceeded to study combinations of colored flashes with colored backgrounds.

2. VARIATION OF UNIT SIZES WITH THE COLORS OF FLASHES AND OF BACKGROUND LIGHTS

The combination of colors was suggested by our earlier finding of Emerson effects in a study on quantum requirements (41). In the following we have blue flashes on darkness, on red, blue, and far-red backgrounds, and yellow flashes on blue and far-red backgrounds. For the experiments summarized in Figs. 8–11 we used leaves of John Williams Broadleaf and Su/su which,

according to our experience, were likely to produce the smallest units under our standard condition of white flashes on red background.

There is little to add in words to what these figures make obvious. Presence or absence of far-red background light determined how efficient a saturating flash could be. In blue light alone small units were hard to find. In the combination of blue flash with far-red background the small units reappeared,

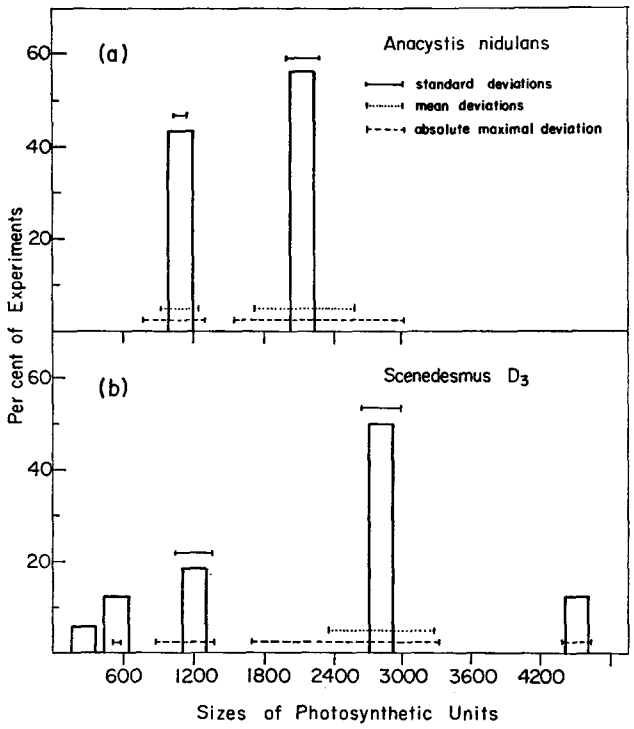


FIGURE 7. (*a*) *Anacystis nidulans* (Richter), age 4–13 days after inoculation from liquid subculture; average age 8 days. 23 flashed samples, 23 controls. Chlorophyll per assay was varied between 4.6 and 48 γ total chlorophyll. (*b*) *Scenedesmus obliquus* D_3, age 4–13 days after inoculation from slant, average age 8 days. 16 flashed samples, 16 controls. Chlorophyll per assay was varied between 6 and 80 γ.

and when the plants were of the chlorophyll-deficient aurea type they became predominant. In Fig. 11 a simple shift from either no background (during flashes) or from a blue to a far-red background made the standard size units of 2400 disappear completely in favor of those of 300 and 600. In other words, the Emerson effect improves not only a suboptimal quantum efficiency at low light, but also the efficiency at flash saturation. One test with saturating yellow flashes on either blue or far-red background (Table II) also showed the superiority of far-red over blue background, but the yellow flash itself may have con-

tained sufficient far-red to eliminate any difference that a far-red background would have made for the chlorophyll-rich John Williams Broadleaf plant. The data of Table II give, by the way, a good example of the few cases in which we found a forbidden size for units—namely 1800—a result to be expected if about half of the size 1200 units had already doubled to size 2400.

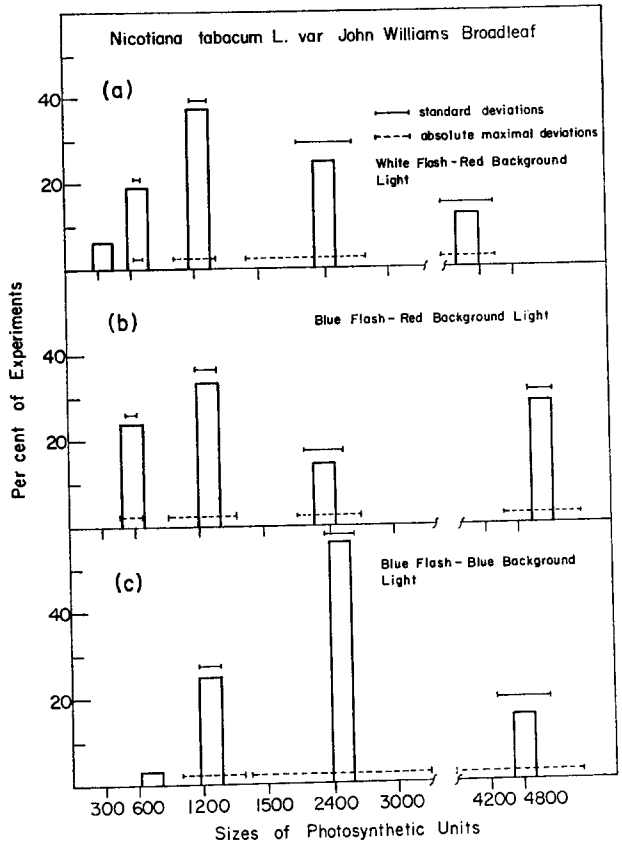


FIGURE 8. *Nicotiana tabacum* L. var. John Williams Broadleaf, young plants 6–8 wk old. (*a*) 350 white xenon flashes on 900 ergs cm^{-2} sec^{-1} of red background light $575 < \lambda < 700$ mμ. 16 flashed samples, 33 controls. Leaf area per assay 2.25 cm^2. 49 chlorophyll determinations; highest value 37.3 γ/cm^2; lowest value 8.6 γ/cm^2; average value 25.6 γ/cm^2; standard deviation $\pm$ 0.77 γ/cm^2. (*b*) 350 blue xenon flashes on 900 ergs cm^{-2} sec^{-1} of red background light as in (*a*). 21 flashed samples, 33 controls. Leaf area per assay 2.25 cm^2. 54 chlorophyll determinations; highest value 54.8 γ/cm^2; lowest value 13.9 γ/cm^2; average value 24.2 γ/cm^2; standard deviation $\pm$ 0.81 γ/cm^2. (*c*) 350 blue xenon flashes on 540 ergs cm^{-2} sec^{-1} of blue background light, $380 < \lambda < 575$ mμ. 32 flashed samples, 36 controls. Check for flash saturation: smallest unit with 50% blue flash intensity 1080 chlorophylls/CO_2 fixed/flash. 68 chlorophyll determinations in 1.5 cm^2. Highest value 58.7 γ/cm^2; lowest value 11.1 γ/cm^2; average value 27.7 γ/cm^2; standard deviation $\pm$ 0.86 γ/cm^2.

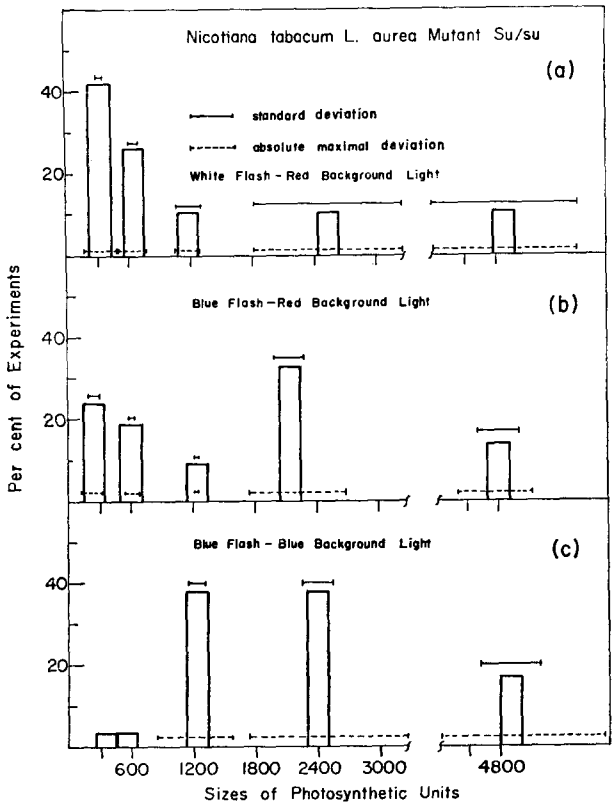


FIGURE 9. *Nicotiana tabacum* L. aurea mutant Su/su, young plants 6–8 wk old. (*a*) 350 white xenon flashes on 900 ergs cm^{-2} sec^{-1} of red background light $575 < \lambda < 700$ mμ. 19 flashed samples, 30 controls. Leaf area per assay 3 cm^2. 49 chlorophyll determinations: highest value 12.2 γ/cm^2; lowest value 1.2 γ/cm^2; average value 6.6 γ/cm^2; standard deviation $\pm$ 0.32 γ/cm^2. (*b*) 350 blue xenon flashes on 900 ergs cm^{-2} sec^{-1} of red background light as in (*a*). 21 flashed samples, 35 controls. Leaf area per assay 3 cm^2. 56 chlorophyll determinations: highest value 13.6 γ/cm^2; lowest value 1.7 γ/cm^2; average value 6.2 γ/cm^2; standard deviation $\pm$ 0.33 γ/cm^2. (*c*) 350 blue xenon flashes on 540 ergs cm^{-2} sec^{-1} of blue background light. 29 flashed samples, 42 controls. Controls consisted principally of checks on flash saturation. Lowest unit obtained with 50% of blue flash intensity was 970 molecules of chlorophyll/molecule CO_2 fixed/flash. 71 chlorophyll determinations. Highest value 14.1 γ/cm^2; lowest value 5.7 γ/cm^2; average value 7.53 γ/cm^2; standard deviation $\pm$ 0.22 γ/cm^2.

3. INDUCTION PHENOMENA

The manifold phenomena which are known to occur in the photosynthetic systems during the transitions from dark to light or vice versa, usually called induction periods and aftereffects, are so complex—as the extensive literature attests—that it has been our aim to circumvent or to erase them as far as possible.

But two kinds must be mentioned because they can considerably alter the outcome of experiments with flashes and provide further insight into the working of far-red illumination. When we had just begun our studies of photosynthetic units we published a figure showing two very contrasting results (39) in respect to the effects of the first few flashes. With the aurea mutant, the

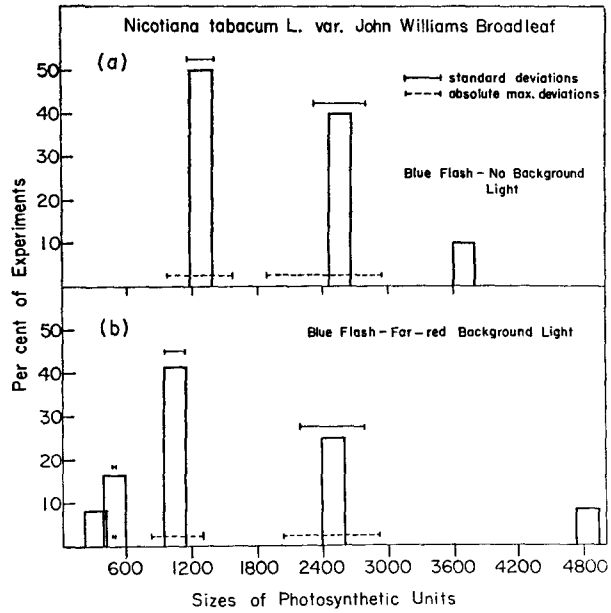


FIGURE 10. *Nicotiana tabacum* L. var. John Williams Broadleaf, young plants 6–8 wk old. (*a*) 350 blue xenon flashes. No background light. 10 flashed samples, 10 controls. 20 chlorophyll determinations in 1.5 cm^2. Highest value 29.2 γ/cm^2; lowest value 15.3 γ/cm^2; average value 22.4 γ/cm^2; standard deviation $\pm$ 0.93 γ/cm^2. (*b*) 350 blue xenon flashes on 6600 ergs cm^{-2} sec^{-1} of far-red background light $700 < \lambda < 750$ mμ. 12 flashed samples, 12 controls. 24 chlorophyll determinations in 1.5 cm^2. Highest value 31.2 γ/cm^2; lowest value 16.9 γ/cm^2; average value 22.6 γ/cm^2; standard deviation $\pm$ 0.78 γ/cm^2.

first two or three flashes after a dark pause fixed an unbelievable amount of carbon dioxide, while in the same vessel the dark green John Williams Broadleaf or *Cassia* gave no fixation at all for the first half dozen flashes, this in spite of a white background illumination of 2700 ergs cm^{-2} sec^{-1}. The one point in which the experiments differed from those reported above was the long dark times of 5 sec between the 3 μsec flashes (air flashes). The latter observations could later be easily confirmed and extended. In experiments with single flashes spaced 5 sec apart, the first 30 flashes usually gave irregular results. The absence of CO_2 fixation could develop even further into a loss of fixed CO_2—in other words, into a carbon dioxide gush which arose from material produced a

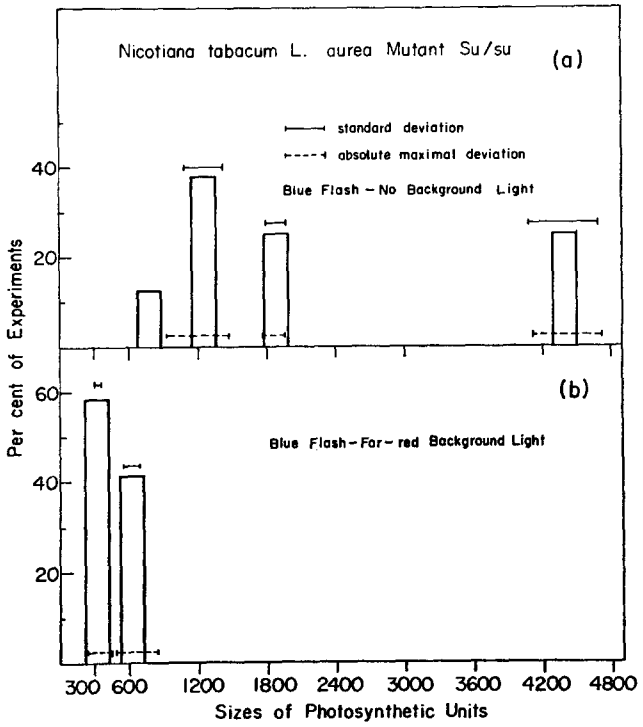


FIGURE 11. *Nicotiana tabacum* L. aurea mutant Su/su, young plants 6–8 wk old. (*a*) 350 blue xenon flashes. No background light. 8 flashed samples, 11 controls. 19 chlorophyll determinations in 3 cm^2. Highest value 10.1 γ/cm^2; lowest value 3.8 γ/cm^2; average value 6.5 γ/cm^2; standard deviation $\pm$ 0.5 γ/cm^2. (*b*) 350 blue xenon flashes on 6600 ergs cm^{-2} sec^{-1} of far-red background light $700 < \lambda < 750$ mμ. 12 flashed samples, 11 controls. 24 chlorophyll determinations in 3 cm^2. Highest value 11.4 γ/cm^2; lowest value 2.7 γ/cm^2; average value 6.7 γ/cm^2; standard deviation $\pm$ 0.45 γ/cm^2.

TABLE II

PHOTOSYNTHETIC UNITS AS MOLECULES OF CHLOROPHYLL PER MOLECULE CO_2 FIXED PER FLASH

Light	*Nicotiana tabacum* L. aurea mutant Su/su	*Nicotiana tabacum* L. John Williams Broadleaf
Yellow flash Blue background light	1205±105	1793±164
Yellow flash Far-red background light	747±55	1747±84

Age of plants: aurea mutant 8 wk old; chlorophyll content 10.2 $\pm$ 0.35 γ/cm^2; highest value 14.1 γ/cm^2; lowest 8.3 γ/cm^2.
Green control: growing leaves of adult plants. Chlorophyll content 36.8 $\pm$ 0.95 γ/cm^2; highest value 42.1 γ/cm^2; lowest value 32.1 γ/cm^2. Xenon flashes filtered through orange plastic filter.

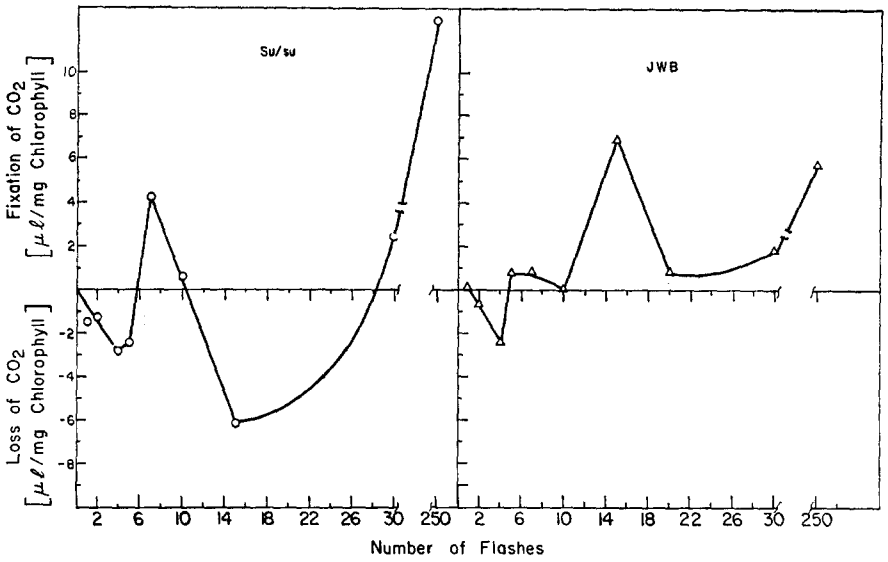


FIGURE 12. Induction phenomena with single xenon flashes on 900 ergs cm^{-2} sec^{-1} of red background light. 17–18°C. Average of seven experiments.

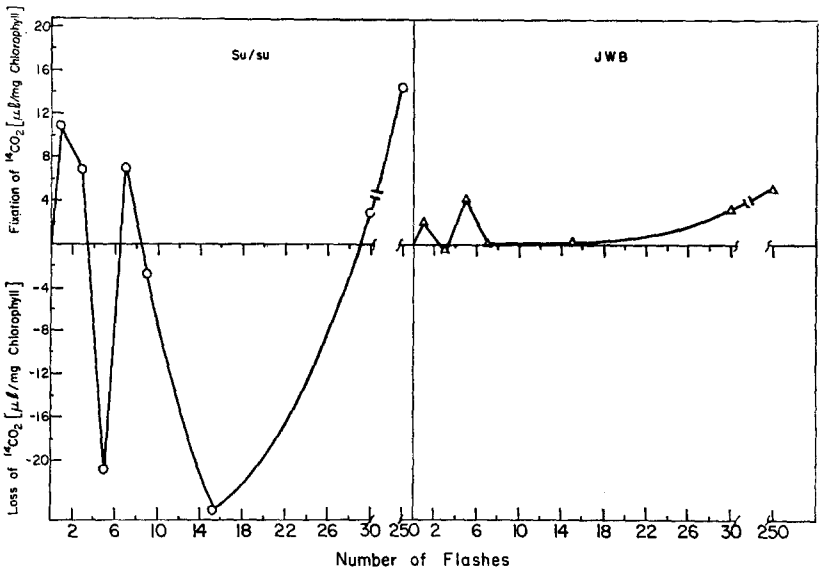


FIGURE 13. Induction phenomena as in Fig. 12. Single experiment. (*a*) Su/su. The fixation of 11 μl CO_2/mg chlorophyll/flash corresponds to 2 chlorophyll per 1 CO_2 fixed per flash for the yellow tobacco. This was the highest value ever observed. On the average the efficiency within the first 10 flashes never exceeded 40 chl/1 CO_2 fixed/flash. (*b*) Green control. Here the extreme case was 10 chl/CO_2 fixed in the first flash. Otherwise the efficiency was never better than 50 chl/1 CO_2 fixed/flash.

few minutes earlier during a steady illumination at low intensity. In accord with the greater capacity of aurea mutants at saturating light intensities—either in continuous or in flash illumination—we noted a flash-induced fixation as high as one CO_2 per two chlorophyll molecules in some leaves of this

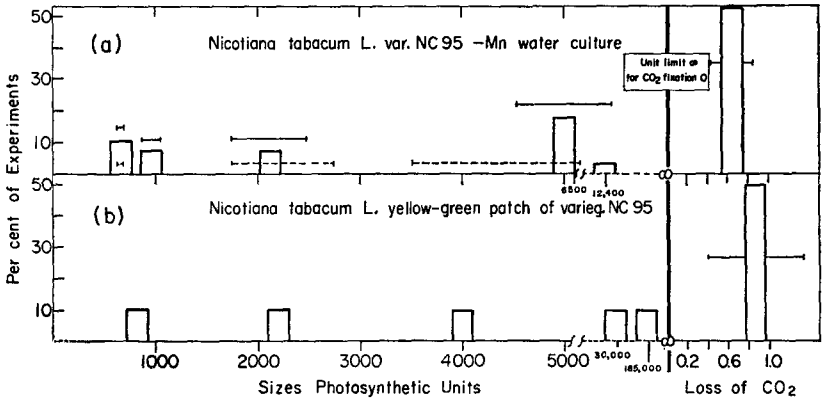


FIGURE 14. 350 white xenon flashes on 900 ergs cm^{-2} sec^{-1} of red background light. Loss [μmoles/g chlorophyll/flash]. (*a*) *Nicotiana tabacum* L. var. NC 95. Plants grown for Mn deficiency in −Mn water culture. 28 flashed samples, 22 controls, leaf area for the assay 3.0 cm^2. 50 determinations of total chlorophyll in 3 cm^2. Highest value 17.5 γ/cm^2; lowest value 2.9 γ/cm^2; average value 10.4 γ/cm^2; standard deviation ± 0.54 γ/cm^2. (*b*) *Nicotiana tabacum* L. yellow patch of variegated NC 95. 10 flashed samples, 2 controls. 12 determinations of total chlorophyll in 3 cm^2. Highest value 14 γ/cm^2; lowest value 1.1 γ/cm^2; average value 5.6 γ/cm^2; standard deviation ± 1.38 γ/cm^2.

TABLE III

EFFECT OF MANGANESE OR VANADIUM DEFICIENCY ON THE PHOTOSYNTHETIC UNIT IN DIFFERENT ALGAE

Algal species	No. of experiments	Fixation of CO_2	Photosynthetic unit
		μmoles/g chlorophyll/flash	*molecules chlorophyll/molecules CO_2 fixed/flash*
Normal culture *Ankistrodesmus braunii* 202-7C	7	0.8±0.215	1250
−Mn culture *Ankistrodesmus braunii* 202-7C	8	−0.28±0.11	Negative
	4	0.17±0.049	6000
Normal culture *Scenedesmus* D_3	3	0.45±0.133	2220
−Mn culture *Chlorella vulgaris* 211-8m	1	0.054	18,518
−Vanadium culture *Scenedesmus* D_3	3	0.097±0.06	10,300

350 white xenon flashes on 900 ergs cm^{-2} sec^{-1} red background light.

plant. On the average, the very first flashes caused the plant to take up one CO_2 per 40 molecules of chlorophyll—that is up to two orders of magnitude more than during the rest of the flashing period. Most of these induction phenomena could be abolished by the described pretreatment with far-red light.

Though initial CO_2 losses from saturating flashes could be seen in perfectly healthy plants, they were very accentuated in those plants which suffered from a diminished ability to evolve oxygen, particularly in two classes—leaf tissues of manganese-deficient tobacco and the yellow patches from variegated tobacco plants which we knew had a lamellar structure deficient for system II reactions. In Fig. 14 we have registered units up to 5000 because such units occur fairly regularly and appear to arise from a doubling of the 2400 unit. Beyond that it makes little sense to talk about units. Less fixation of CO_2 per flash and chlorophyll simply denotes an increasingly defective mechanism. The important thing is that concentrated light energy not used for fixation may lead to the decarboxylation of previously synthesized compounds (Table III). The same phenomenon has been recently described for algae by Miyachi (28, 29).

DISCUSSION

For thirty years after the discovery of Emerson and Arnold the unit and the problem of energy transfer have been the subject of ever recurring theoretical discussions (8, 12, 16, 27, 36, 47). The results reported here leave no doubt that a unit in the sense of a single, fixed chlorophyll structure containing between 2 and 3000 chlorophyll molecules assembled around one reduction center and characteristic for photosynthesis in all green plants, does not exist. Once we accept this conclusion quite a few other observations on "abnormal" flash saturation values for oxygen evolution fall into place (22). And so do the small units found for partial reactions and the metabolism of purple bacteria (6). On the other hand, we are not permitted to say that chlorophyll units have only a statistical existence and are just a measure of the average distance the energy of an absorbed light quantum has to travel through a condensed chlorophyll phase until it hits a reaction center (34, 43). The flash saturation values for CO_2 fixation do not vary continuously but in steps measuring 2, 4, 8, and 16 times a basic unit of about 300. We have found no other way to explain this except by preserving the notion of fairly rigid structural entities, which do not grow by simple addition but by doubling (31, 45). Our first observation on the variability of the units published two years ago posed problems which we believe we have solved by the tedious expediency of large numbers of experiments and controls. We wanted to be able to give more than just qualitative observations on the variability of units. As in the question of quantum yield, it is unimportant whether the smallest chlorophyll unit

which is able to release one O_2 corresponds to 250, 300 or 350 chlorophyll molecules, or whether the multiples are exactly 2, 4, 8, or 16 times as much. What matters is the reality of such distinct steps. The investigator who would be satisfied with only half a dozen measurements would easily come across numbers such as those shown in Table II. This table contains nothing but forbidden numbers, which fall in between the five groups shown in Figs. 4–11. Not only were many experiments needed for statistical evaluation, but also the living material had to be selected. Units vary not only with the combination of spectral colors, but also and much more easily with the internal factors of the growing cells which we have not yet learned to control.

The chlorophyll unit has always been a measure for the inefficiency of flash fixation as compared with photosynthesis in weak light. As we have shown, the low yield can get worse until it turns into flash-induced losses of fixed material (Fig. 14). Only constant yields per flash can tell us something about the structure and working mechanism in the chloroplasts.

Thirty years ago H. Kohn pointed out that the unit need not be measured in terms of $+O_2$ or $-CO_2$ per 2400 chlorophyll molecules but just as well and perhaps more realistically in terms of those reduced intermediates of which several contribute to the final result (26). Energy transfers would have to be considered within sets of 600 chlorophyll molecules per reducing [H] corresponding to two photoacts, or, according to Baur's old, recently revived terminology of charge separation into (+) and (−) (3), in sets of 300 per electron transfer per one quantum absorbed. Now, since we know that 2400 is only one among several possible numbers, it is perhaps more convenient and more meaningful to convert old type units on the basis of eight primary processes into new type units by dividing by eight, or better still to adopt some measure for the efficiency of one flash. In this way we obtain the following conversion table:

Old type U	320	640	1280	2560	5120
New type U	40	80	160	320	640
Flash efficiency (FE)	1	0.5	0.25	0.12	0.06

The FE is a measure of the number of available reaction centers in terms of the smallest chlorophyll unit. A high FE correlates with high saturation rates in continuous illumination, the supply of the products induced by near infrared, and good quantum efficiencies.

In view of our results we may now say that the efficiency of a saturating flash is but a measure of the probability that the number of electrons which appear simultaneously in system I and system II match each other and cooperate perfectly to give either oxygen evolved or carbon dioxide fixed.

A nearly perfect match will happen only if there are no bottlenecks anywhere in the over-all electron transport system, and if the distribution of

quanta absorbed by different pigment systems is such as to produce the proper ratio of various primary products. One of the puzzles discussed many years ago was the coexistence of a very high quantum efficency with such a low flash efficiency (16, 47).

This puzzle disappears when we consider that at a fairly low steady illumination used for quantum yield determinations none of the pools of intermediates in the chain will be either entirely reduced or oxidized or "activated". All these pools will contain a sufficient fraction of their molecules in the right state to allow for an uninhibited progress via all intermediary steps as long as the rate is low. But the sudden appearance of a great number of primary electrons and "holes" in all active pigment units cannot be taken care of in the best possible manner unless at the moment of the flash all reaction centers as well as all pools of intermediates are in a rather one-sided state and therefore ready to receive a surge of primary products. And this optimal state is what far-red light is apparently able to bring about.

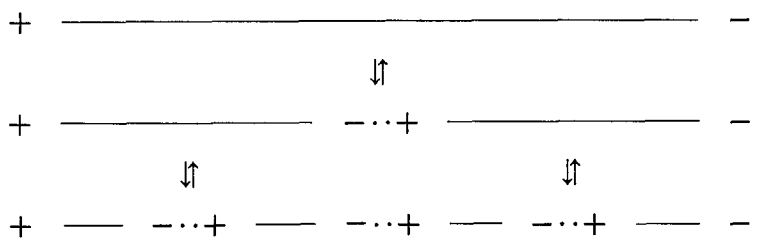
Homann and Schmid found earlier that in chloroplasts of Su/su the saturation rate of the ferricyanide Hill reaction was unusually high (19). They concluded that either the turnover time of the enzymatic system had to be shorter or the PSU smaller. Fig. 4 shows that the average sizes of photosynthetic units in the aurea plants are indeed smaller than those of the green John Williams Broadleaf. They also found that phosphorylation mediated by system I occurs in single, unfolded frets. The difference in the results for John Williams Broadleaf and Su/su in our Figs. 8–11 might be explained by the high ratio in Su/su chloroplasts of unfolded fret areas to the partition areas (doublings, "grana").

No combination of properties so far attributed to the PSU in thirty years of discussion suffices to explain our experimental findings. Statistical variations of the average distance between the light-absorbing molecules and reaction centers in growing chlorophyll clusters do not explain numerically related group values. The most surprising result was that 15 min of preillumination with far-red light at the right time may often change the size of nearly all units.

We have as yet not elaborated a detailed theory as to how the groups of units and this power of infrared light can be fitted smoothly into the modern picture of the photosynthetic mechanism. As a starting point we offer, however, a very simple scheme which makes use of the fact that in the living world nearly everything grows by duplication and hence by the powers of two, and that active membranes or structures show polarity.

The following three lines represent the cross-section of a two dimensional chlorophyll unit in successive states of changing from low to high FE. + and − at the ends of the first line signify corresponding reaction centers which give rise to electrons or holes respectively. A new pair of $-\cdots+$ which appears first in the middle of the second line means that the large unit has been bisected

by a new string of reaction centers. This structural change may repeat itself until the smallest unit with the highest FE has been reached.



The most efficient structure is maintained only as long as the pigment characterized by its absorption in the far-red delivers the necessary energy. The over-all orderliness may come from an underlying matrix which contains this very pigment. The sketch can be turned 90° and made to look like venetian blinds, meaning cross-sections of chlorophyll layers with alternating layers of reaction centers and their enzymes. The latter disappear whenever two layers of light-harvesting chlorophyll fuse to give a double-sized unit with half the numbers of reaction centers.

System I and system II are supposed to have separate units. They ought to have independent effects on the FE. We mention this only to point out what further problems in constructing unit models can easily be envisaged. That energy-rich substances—for which ATP or its precursors are the prototype—may play a decisive role in the formation or dissolution of microstructures is not a new thought at all (2, 10, 11, 20, 46). For several years we have pointed out the advantage of having a pigment which serves solely as a producer of ATP and is not directly engaged in the oxido-reduction process (15, 24).

Finally we must say a few words about the abnormally high or low fixation values found with isolated flashes. If in the time before a flash a chloroplast contains a slight excess of ribulose monophosphate and some reduced ferredoxin or reduced NADP but no ATP, illumination will preferentially induce ATP formation (4, 14), particularly in chloroplasts structured like those of the aurea mutant (19) where single frets abound in comparison with lamellar doublings or grana. Under these conditions a flash will cause the fixation of small amounts of carbon dioxide with as little energy as is needed for photophosphorylation. The opposite effect, lack of fixation by dark green tissue, has been known much longer. In the presence of phosphate acceptors or of internal natural Hill reagents (quinones?), CO_2 fixation may easily occur only after a considerable delay (13). Finally a partial inhibition of the reactions on the path to oxygen may result in a decarboxylation of some products of photosynthesis (malate?) in place of the normal CO_2 fixation (23).

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