Photosynthesis

Experiments at the Max Planck Institute for Cell Physiology, Berlin-Dahlem, 1950–57, are described.

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Ever since *Chlorella* has been an object of photosynthetic study, it has been known that there are cells that use light efficiently and cells that use light inefficiently. In recent years we have sought to discover and control the conditions that give rise to efficient cells. It has been found that one of the most important conditions is the light intensity at which the cells are cultured. If one employs artificial light sources without interruption, as has been the almost universal practice, the *Chlorella* are then too far removed from their natural living conditions of the past half billion years. The cells are forced to produce organic matter continuously, and more material than they need for their own synthesis. As a consequence, the energy yield of the cells is reduced to a small fraction of the optimum yield.

Cells that use light efficiently result, on the other hand, when one allows the intensity of the light to fluctuate so as to imitate day and night, with dimming late evening and early morning (*1*). We attain this by varying the operating voltage automatically from 50 up to 220 volts and back to 50 volts again over a period of 24 hours. The relative quantum intensities of radiation were measured with the chemical quantum actinometer (*2*), with results indicated by the ordinate values in Fig. 1. Cells so cultured use the light best when they are placed in the manometric vessels in the morning and their photosynthetic efficiency is measured thereafter during the artificial day.

Equally as important as the culturing of the cells are the conditions under which the utilization of the light is measured. For example, it was found with monochromatic light that the utilization of light in the green or yellow or red was the poorer the purer the spectral composition. However, good utilization was immediately restored when a relatively small amount of blue-green light was added to the main beam of very pure monochromatic light. One can thus obtain good or poor yields at will, simply by adding or removing the blue-green light during the measurements of efficiency. If each such test period is made 30 minutes long, one can observe in an experimental day of 8 hours, with one and the same suspension, good yields eight times and poor yields eight times!

The different parts of the blue-green spectrum are not equally effective. The action spectrum of the blue-green light shows a sharp maximum in the region of 460 mμ, as is shown in Fig. 2. This action spectrum is probably a carotinoid spectrum. An inactive carotinoid proenzyme is probably converted by the blue-green light into an active lumino enzyme. As possible analogs, there may be mentioned light-sensitive visual purple, and ooverdin, a carotinoid protein discovered by Richard Kuhn.

Both examples—the fluctuating light during culture and the blue-green light during yield measurement—suffice to make it understandable why, in the last 40 years, in different institutes throughout the world, very different photosynthetic yields have been found—different not in percentages, but in hundreds of percent. Even if the manometry and the light measurements had been correct everywhere, not even approximate agreement would have been possible, owing to ignorance of the essential conditions of culture and measurement. Thus, in the United States, during the years 1938 to 1948, an average quantum requirement of 16 per molecule of oxygen gas produced was found, corresponding to an energy yield of 18 percent in red light. This value is removed from the optimal value (*1*) by several hundred percent.

If one maintains the now-established conditions of good yield, one will obtain good yields from now on, everywhere and always. Figure 3 shows an example of oxygen evolution during constant illumination in a 5-hour experiment in which the quantum requirement per molecule of O_2 produced was approximately 3 for the entire period. Any deviation from linearity with time was within the experimental error. Figure 4 shows oxygen development in a 6-hour experiment in which the quantum requirement per molecule of O_2 produced was approximately 4. Table 1 contains the results of 23 six-hour experiments conducted on 23 days of the months March to May, 1957, in which only a single instance of a poor yield, namely a quantum requirement of 7.5, was obtained (*3*).

The quantum requirement of 3 per molecule of O_2 signifies that in red light about 90 percent of the incident light energy can be converted into chemical energy. Since light energy is freely transformable energy, this energy efficiency is completely compatible with both the first and second laws of thermodynamics. Thermodynamically incompatible with good yields were only those theories concerning the chemical mechanism of photosynthesis that are today at long last recognized as incorrect.

In summary, one can say that, with the fixing of the conditions of culture and measurement, the dispute concerning the efficiency of utilization of sunlight is finally decided. It is a decision in favor of nature. The reaction by which nature transforms the energy of sunlight into chemical energy, and upon which the existence of the organic world is based, is not so imperfect that the greater part of the applied light energy is lost; on the contrary, the reaction is, like the world itself, nearly perfect.

The Multiquanta Problem

But how is it possible that carbonic acid can be split by the light quanta of visible light, which are so deficient in energy that several quanta are necessary? In the photochemistry of the inanimate world, no reactions are known in which several quanta react with *one* molecule at one time, and, moreover, several-quanta reactions are theoretically scarcely conceivable.

The problem was solved several years ago at Dahlem by Dean Burk and us (*4*).

Professor Warburg is director of the Max Planck Institute for Cell Physiology, Berlin-Dahlem, Germany. This article is based on a lecture he delivered in Berlin on 5 Oct. 1957 before the Society of German Chemists. It was first published, in German, in *Angewandte Chemie* [69, 627 (1957)], in collaboration with W. Schröder, G. Krippahl, and H. Klotzsch. This translation was prepared by Dean Burk and George Hobby, National Institutes of Health, U.S. Public Health Service, Bethesda, Md., with the permission of *Angewandte Chemie* and the approval of Professor Warburg.

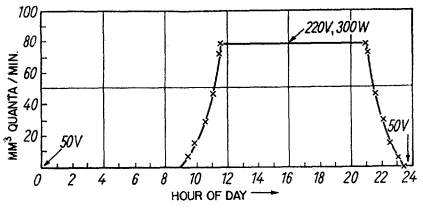

Fig. 1. Fluctuating light intensity in the culturing of *Chlorella*.

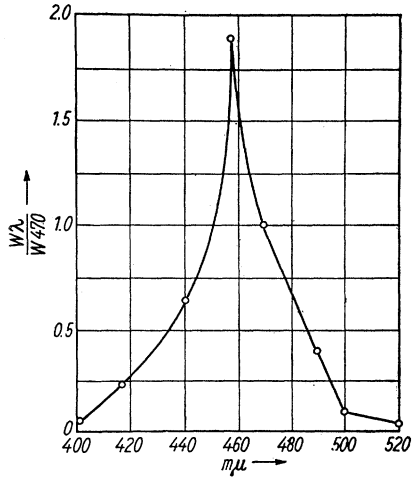

Fig. 2. Action spectrum of blue-green light.

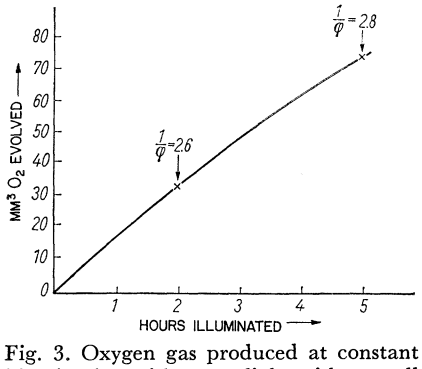

Fig. 3. Oxygen gas produced at constant illumination with green light with a small quantity of blue-green light added (five-hour experiment).

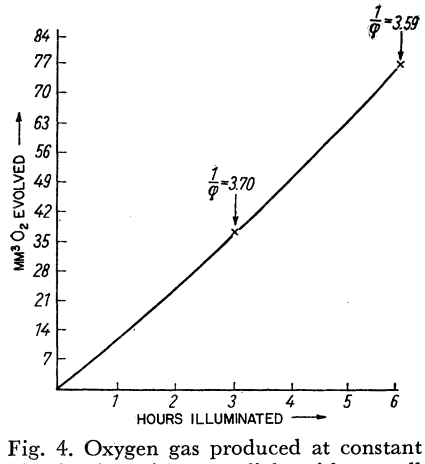

Fig. 4. Oxygen gas produced at constant illumination with green light with a small quantity of blue-green light added (six-hour experiment).

By measuring photosynthesis under special conditions, a splitting of photosynthesis into two reactions was observed: a *light reaction* and a *dark reaction*. Normally these two reactions overlap each other so that one cannot observe each one separately.

In the light reaction, one molecule of O_2 will develop per molecule of chlorophyll, with, however, a quantum requirement, not of 3, but of 1. This at first appears to contradict the laws of energy. However, during the dark period following the end of illumination it can be observed manometrically, under suitable conditions, that two-thirds of the oxygen gas developed during the light period undergoes a back reaction, with restoration of the original condition wherein light can again produce O_2 as before (*5*). Thus, if the light reaction is not considered by itself, but together with the dark reaction, all is in order energetically.

Closer study showed that in the dark reaction the oxygen of carbonic acid was so loosened that, with the help of the energy of respiration, one quantum then sufficed to produce one molecule of O_2. The carbonic acid derivative with the loosened oxygen is probably a peroxide. In order not to go beyond the facts, we may call it the "photolyte" of photosynthesis.

If we write the light and dark reactions of photosynthesis one after the other, we obtain (Chl, chlorophyll):

$$\text{(Light)}\quad 3(\text{ChlCO}_2{}^*) + 3\text{N}_0h\nu + 3\text{CO}_2 \rightarrow 3(\text{ChlCO}_2) + 3\text{C} + 3\text{O}_2 \quad (1)$$

$$\text{(Dark)}\quad 2\text{C} + 2\text{O}_2 \rightarrow 2\text{CO}_2 + 200{,}000 \text{ cal} \quad (2)$$

$$\text{(Dark)}\quad 3(\text{ChlCO}_2) \rightarrow 3(\text{ChlCO}_2{}^*) - 200{,}000 \text{ cal} \quad (3)$$

$$\text{(Balance)}\quad 1\text{CO}_2 + 3\text{N}_0h\nu \rightarrow 1\text{C} + 1\text{O}_2$$

The photolyte derivative of carbonic acid is designated by an asterisk, in order to distinguish it from the untransformed carbonic acid. Nothing in this reaction sequence is theory. All has been found experimentally and measured in *living Chlorella*. Reaction 1, the *light reaction*, is measured by the O_2 development and CO_2 consumption in the light. Reaction 2 is measured by the O_2 consumption and CO_2 production in the dark. Reaction 3, in which the bound, inactive carbonic acid is transformed into the photolyte, is measured by the time that elapses until the light is again able to develop as much O_2 as in reaction 1. In our experimental arrangement this recovery period for full light action lasted about 20 minutes, and could therefore be followed very accurately in its time course.

Table 1. Quantum requirement in 23 consecutive experiments (mole quanta absorbed by chlorophyll/mole O_2 developed).

Date (1957)	Quantum requirement
Mar. 1	4.10
3	3.68
5	3.65
6	3.58
7	4.30
14	3.65
21	4.10
22	3.22
25	4.61
27	3.90
29	3.56
Apr. 1	3.49
3	4.75
9	4.26
10	4.65
11	4.30
15	7.51*
17	3.92
23	3.54
24	2.92
25	3.20
26	4.62
May 1	3.90

* This was the single instance of a poor yield.

The photolyte is written as a chlorophyll compound because the quantity of O_2 that the light can develop from the photolyte is equivalent to the chlorophyll content of the cells (*6*). This is important. We now no longer have any need to wonder how it is possible that the light energy is transferred without loss from the chlorophyll molecule to the photolyte molecule, since we now know that the light acts within the same molecule that absorbs it. The light reaction of photosynthesis is thus nothing else than the photodissociation of a pigment, comparable to the photodissociation of carbon monoxide-hemin compounds, and the quantum yield of 1 is almost self-evident.

Upon adding the three equations of the reaction sequence, the photolyte is eliminated, and the net result is the splitting of carbonic acid by 3 quanta of light, which is what one finds experimentally in the balance of photosynthesis.

Nothing seems to be simpler than this solution of the quantum problem. Of the 110,000 calories that are necessary for the splitting of 1 mole of carbonic acid, 70,000 are provided by a respiratory process. The remaining 40,000 calories that the light provides is exactly the amount of energy of 1 mole quanta of red light. All quantum difficulties are thus eliminated.

In order fully to appreciate this solu-

tion, one must reflect that in photosynthesis no energy would be gained, but rather lost, if the energy of the respiratory process were taken from the energy store of the cells. Only because the respiratory energy of reaction 2 is directly supplied by light is a net gain of energy attained. All detail is simple physics and chemistry. But the whole is a higher kind of physics and chemistry, devised by the genius of living nature.

To conclude the discussion of energetics, I would like to describe an experiment that ought to be demonstrated to all students of biochemistry, because it confirms in the simplest possible way the requirement of our equations that there is no photosynthesis without respiration.

Figure 5 shows the experimental vessel that is to be attached to a manometer. The vessel contains *Chlorella* suspended in a carbonate-bicarbonate mixture that maintains the CO_2 pressure constant, so that pressure changes registered by the manometer can only be changes in O_2 pressure. The gas space contains argon and a very little oxygen.

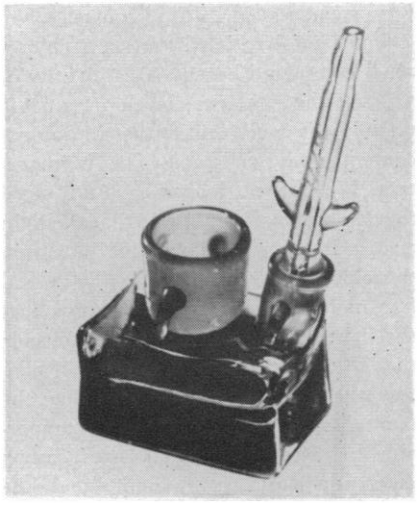

Fig. 5. Vessel for demonstration of the necessity of respiration for photosynthesis.

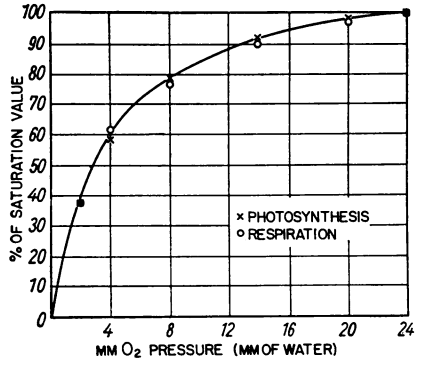

Fig. 6. Respiration and photosynthesis at low pressures of O_2.

The essence of our experimental arrangement is that we employ the cells themselves to attain the desired low O_2 pressures. When we darken the cells the O_2 pressure sinks at once on account of the respiration; and when we illuminate the cells the O_2 pressure rises at once on account of the photosynthesis. This cycle can be repeated as often as desired without opening the vessel. The manometer shows us at any time the prevailing O_2 pressure, and the change in manometer fluid level shows us for any time period of pressure change the respective respiration or photosynthesis. We thus learn whether, and in what manner, respiration or photosynthesis changes as a function of O_2 pressure.

The result is shown graphically in Fig. 6, in which the changes of respiration and photosynthesis are plotted against O_2 pressure. As one sees, both respiration and photosynthesis change with O_2 pressure, and indeed identically. An O_2 pressure of 3 mm of water is the half-saturation value for both processes, and 20 mm yields virtual saturation for both processes. Below an O_2 pressure of 1 mm of water, respiration and photosynthesis are both very small.

The experiment shows much more than that oxygen gas is necessary for photosynthesis. It shows that not merely traces of oxygen are necessary, but definite and easily measurable pressures of oxygen, and that these pressures are necessary because they are necessary for the respiration. All is precisely as our equations demand. *Without respiration, no photosynthesis!*

Chemistry of Photosynthesis

We now leave energetics and turn to the chemistry of photosynthesis. The problems posed here are clearly given by the results of the energetics. What happens chemically to carbonic acid in the dark reaction of photosynthesis? Or, expressed otherwise, what is the photolyte chemically? The gates to this field were opened by the following experiment (7).

The main compartment of a conical manometric vessel (Fig. 7) contains a suspension of *Chlorella,* the side arm contains fluoride, and the gas space contains argon free of CO_2 and O_2. The *p*H of the suspension and of the fluoride is 3.8. Upon tipping the fluoride from the side-arm into the main compartment, a vigorous evolution of CO_2 from the cells takes place. From 100 mm^3 of *Chlorella* cells, 30 to 40 mm^3 of CO_2 will be developed in a few minutes. The content of

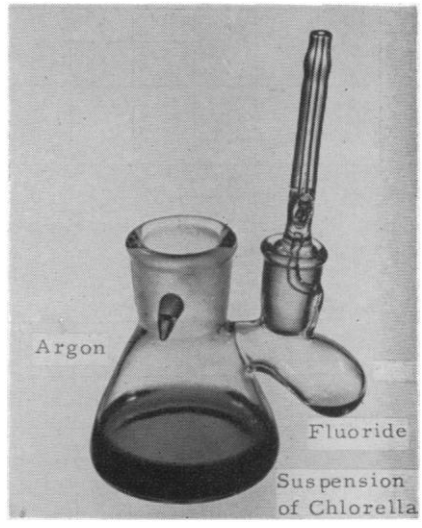

Fig. 7. Vessel for measuring labile CO_2.

this labile CO_2 in *Chlorella* is thus very great—greater, for example, than the content of oxyhemoglobin-O_2 in red blood cells. A trace of cyanide diminishes the development of the CO_2, from which one must conclude that it is an enzymic reaction that is activated by the fluoride.

There are two facts of special interest about the fluoride reaction. First, if one expels the CO_2 with $N/1000$ fluoride anaerobically, and then passes O_2 into the suspension, the expelled CO_2 will for the most part be taken up again. The energy of the respiration induced by the added O_2 is necessary for this rebinding of CO_2. Obviously, the analogy here to the dark reaction in photosynthesis is very far-reaching.

Second, and equally important: if one expels the labile CO_2 from the *Chlorella* with low concentrations of fluoride, and then illuminates, photosynthesis is found to be inhibited; but if one removes the fluoride from the cells by washing, and waits until the CO_2 is again aerobically bound, the photosynthetic capacity is found to be restored. Labile CO_2 and photosynthesis are thus mutually dependent.

We have spared no pains to discover what the chemical source of the labile CO_2 is. We have found that it is L-glutamic acid (*8*), which occurs in *Chlorella* in loosely bound form to the extent of 0.5 to 1 percent of the dry weight. This glutamic acid goes into the external medium when a *Chlorella* suspension is heated at 90°C for several minutes. If one determines the glutamic acid content of the centrifuged external medium *before* and *after* a treatment with fluoride, one finds that as much glutamic acid has disappeared as CO_2 has been developed by the fluoride!

γ-Aminobutyric acid is formed along with the CO_2 in the fluoride reaction. Aerobically, γ-aminobutyric acid and CO_2 react in the cells to yield glutamic acid again, so that aerobically a stationary state is set up between decomposition and resynthesis of glutamic acid:

L-Glutamic acid ⇌
γ-aminobutyric acid + CO_2

The α-decarboxylation of glutamic acid was discovered in bacteria in 1910 by Ackermann and in green plant cells in 1937 by the Japanese Okonuki. Both the decomposition and resynthesis of glutamic acid can be demonstrated when one transfers the heated extracts of *Chlorella* onto chromatograph filter paper, develops with phenol-citrate solution, and sprays with ninhydrin in the standard way.

As known test substances for the chromatogram, we have employed aspartic acid, glutamic acid, alanine, and γ-aminobutyric acid. The experimental runs in Fig. 8 show that under its normal living conditions *Chlorella* contains little aspartic acid, much glutamic acid, much alanine, and no γ-aminobutyric acid. We presume that the glutamic acid is combined with the chlorophyll, since normally cultured cells contain one to two molecules of glutamic acid per molecule of chlorophyll. In *N*/80 fluoride the glutamic acid decreases and appears as γ-aminobutyric acid. This result is obtained both anaerobically and aerobically, since at this high concentration of fluoride no glutamic acid will be resynthesized.

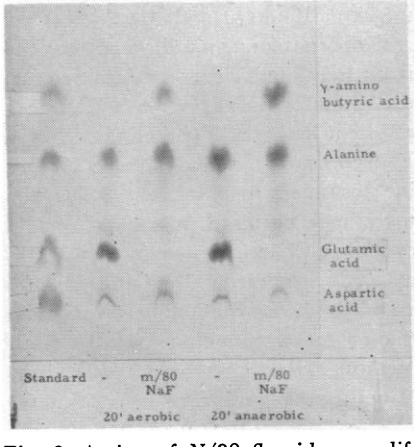

Fig. 8. Action of *N*/80 fluoride: no difference aerobically and anaerobically (phenol-citrate-phosphate; Whatman filter No. 1 unidimensional).

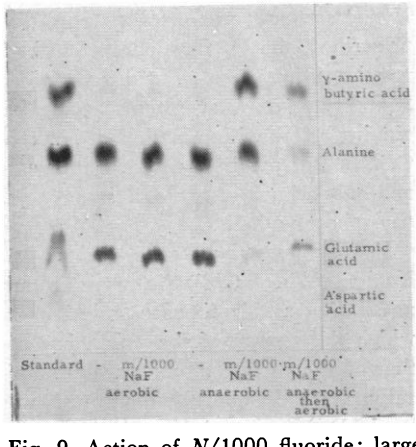

Fig. 9. Action of *N*/1000 fluoride; large difference aerobically and anaerobically (phenol-citrate-phosphate; Whatman filter No. 1 unidimensional).

In the experiment of Fig. 9 the fluoride concentration was only *N*/1000, and here one sees a great difference anaerobically and aerobically. Anaerobically the decomposition is very extensive, but aerobically it is small.

The more the glutamic acid is destroyed, just so much the more is the photosynthesis inhibited. For example, two different concentrations of fluoride were added to aliquot *Chlorella* suspensions under aerobic conditions, and the glutamic acid content and photosynthesis measured. Table 2 shows how closely decomposition of glutamic acid and inhibition of photosynthesis parallel each other.

Continuation of these experiments brought forth a further connection between glutamic acid and CO_2. A study of the binding of CO_2 by *Chlorella* at different pressures of CO_2 showed that the labile CO_2 is not only bound as the α-carboxyl of glutamic acid but in addition an equal quantity of CO_2 is dissociably bound under aerobic conditions. This dissociable CO_2 is also given off when the glutamic acid in the cells is decomposed. The saturation value of the dissociating CO_2 is very nearly equal to the glutamic acid content of the cells. The formation of carbamino-glutamic acid is perhaps involved in the dissociable complex.

In conclusion I may mention further that we have begun to study the behavior of amino acids in photosynthesis with the help of radioactive CO_2. The main compartment of a manometric vessel (Fig. 10) is filled with a suspension of *Chlorella*, the Siamese side arm in one part with C^{14}-carbonate and the other part with excess lactic acid. Upon tipping the acid onto the carbonate, a pressure of radioactive CO_2 is obtained in the gas phase. Vessels so prepared were illuminated ½, 1, or 5 minutes and then, along with dark control vessels, immersed in hot water in order to stop all enzymatic reactions and at the same time extract the soluble materials from the cells. After centrifugation, the heated extracts were chromatographed in two dimensions, and measurements were made with a Geiger counter.

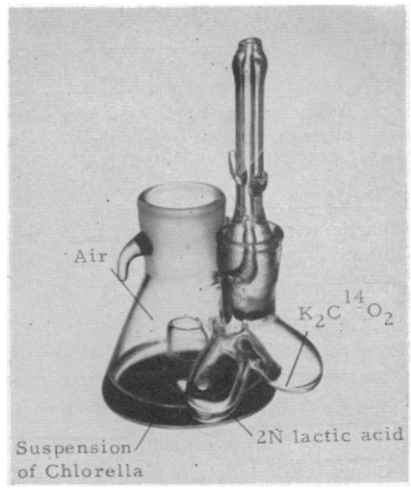

Fig. 10. Manometric vessel for experiments with radioactive CO_2.

It can be seen from Table 3, first, that the amino acids, alanine and aspartic acid, rapidly became radioactive—indeed, more rapidly than the phosphorylated glyceric acid, contrary to previous reports in the literature for experiments of this general type. Second, the table shows that aspartic acid and alanine become radioactive more quickly than glutamic acid, so that one can think that an alanine-aspartic acid system enters before the glutamic acid system. Manometric experiments alone have not as yet given any such indication, and one must wait until combined radiometry, manometry, and bolometry have become so far developed quantitatively that one can draw more certain conclusions. Radiometry alone has already led to a multiplicity of errors.

Summary

With the establishment of conditions for optimum culturing and measurement, there is now final proof that in photo-

Table 2. Comparative action of fluoride on decomposition of glutamic acid and on inhibition of photosynthesis.

Fluoride concentration	Decomposition of glutamic acid (%)	Inhibition of photosynthesis (%)
N/640	21	18
N/320	64	64

Table 3. Geiger counter impulses per minute by chromatographed heated extracts of 10 mm^3 of *Chlorella* cells.

Amino acid or other material	Geiger counter impulses per minute					
	10 min dark, 0.5 min dark	10 min dark, 0.5 min light	5 min dark, 1 min dark	5 min dark, 1 min light	5 min dark, 5 min dark	5 min dark, 5 min light
Aspartic acid	8520	18750	9320	14205	8000	30100
Glutamic acid	1136	1614	1096	1900	3350	17000
Alanine	710	6950	940	19000	817	37500
Phosphorylated sugar and glyceric acid	95	3451	147	9800	523	23800
Nonphosphorylated sugar	Not determined		143	113	857	2726

synthesis at high as well as low light intensities the light energy can be almost completely converted into chemical energy. There is thus drawn to a close an investigation that was initiated many years ago in Berlin in the Imperial Institute of Physics (*9*).

The second result is the establishment of a general physical mechanism of photosynthesis, involving an interplay between light energy and respiratory energy, and therewith the solution of the quantum problem in photosynthesis.

The third result is the establishment of the function of chlorophyll as a stoichiometric, chemically reacting component in photosynthesis.

There remains the special chemistry of photosynthesis. In this still-unfinished field of investigation, the latest discovery is the labile carbon dioxide of *Chlorella,* connected with the decomposition and resynthesis of glutamic acid in living *Chlorella,* and connected with the possible function of the amino acids, aspartic and glutamic, in the binding and reduction of carbonic acid. The dissociating CO_2 is bound by *Chlorella* only in the presence of O_2 and of cellular glutamic acid. This CO_2 is released if the oxygen pressure is lowered below 2 mm of water or if—in the presence of oxygen—the glutamic acid is split in the living *Chlorella,* for example, by *N*/10,000 benzoquinone. This is the CO_2 that is used in light and taken up in the dark (*8*).

Remarks

1) *Quantum requirement in the United States.* In the years 1938 to 1948 the quantum requirement of photosynthesis was measured in various institutes in the United States with the result that 12 to 20 quanta were found to be required by *Chlorella* for the development of one molecule of O_2. The average value was 16. The value of 12 was regarded as the optimum value. A few, including Dean Burk and van Niel, dissented, but the interpreters and advocates of the high quantum numbers, James Franck and Eugene Rabinowitch, maintained the upper hand. In 1941 Franck and Gaffron (*10*) wrote, "*We know now that the high efficiency is only apparent and that the true efficiency is probably only a third of it, namely 12 quanta per molecule CO_2 reduced*" (italics added). The "photosynthetic unit" in Urbana, Emerson and Rabinowitch, stayed with the high quantum numbers until at least 1952 (*11*). Later, under the influence of the Dahlem investigations, the quantum numbers reported in the United States sank, and today approach the Dahlem number of 4 to 3 (*12*).

2) *Light reaction and dark reaction.* According to the equations of the light and dark reactions, which can be separated in point of time, CO_2 is taken up anew in the light reaction, so that the ratio CO_2/O_2 is about -1 in the light reaction per se (although the CO_2 from which the oxygen is developed may not be the CO_2 that is taken up). However, this holds only for optimally cultured cells whose quantum requirement in the balance is 3 to 4. Other cells, for example those grown at a south window with added constant artificial illumination, may take up CO_2 more slowly, so that the ratio CO_2/O_2 in the light reaction is not -1 but lies between -1 and 0. In the latter extreme the new CO_2 is first taken up in the dark reaction following cessation of illumination. Thus there are two reactions of CO_2 to be distinguished: the binding of CO_2, and the transformation of bound CO_2 into the photolyte. In the case of optimally cultured cells, these two reactions of CO_2 may be completely separated, whereas the O_2 development and the binding of CO_2 take place simultaneously and equally. In the less active cells the two reactions of CO_2 are not separated in time, whereas O_2 development and CO_2-binding are separated. The unraveling of these relations has cost many experiments.

3) *The unit of 2500 chlorophyll molecules.* In 1932 Emerson and Arnold (*13*) attempted to apply our methods of intermittent illumination (*14*) to photosynthesis. For example, with very short, very bright light flashes and relatively long dark periods, they determined the maximum quantity of O_2 that appeared to be developed in *one* light flash. Comparison of this quantity of O_2 with the chlorophyll content of the cells showed that 2500 molecules of chlorophyll could develop *one* molecule of O_2. In contrast, we find, without intermittent light, and with direct measurement of the light reaction during inhibition of the dark reaction, that *one* molecule of chlorophyll can develop *one* molecule of O_2. There is thus a discrepancy of three powers of 10, depending upon whether the ratio chlorophyll/oxygen is measured with intermittent light or directly. As Dean Burk (*15, 16*) has shown, however, the intensity of the light flash in the experiments of Emerson and Arnold was several orders of magnitude too low—that is, entirely insufficient—to decompose all the photolyte in the very short time of the light flash ($\sim 10^{-5}$ second).

4) *Burst of carbon dioxide.* According to Emerson and Lewis (*17*), photosynthesis begins with a burst of CO_2 evolution. This phenomenon was discovered manometrically with the two-vessel method, without, however, maintaining the essential requirement of the method. Instead of both vessels being illuminated simultaneously, an interval of 8 to 24 hours took place between illumination of one vessel and the other. This procedure removed the essential condition of the two-vessel method, namely, that in the two unequal vessels the same chemical change must occur. If one properly measures, as nowadays prescribed, the O_2 development in both vessels simultaneously with a divided light beam, one never finds at the beginning of the illumination a burst of CO_2 out of the living cells, but always and only a burst of O_2, corresponding to true photosynthesis.

5) *The experiments of Ruben and Kamen.* When *Chlorella* were illuminated in a bicarbonate solution in which the oxygen of the water, but not that of the carbon dioxide, was isotopically marked, the O_2 developed was marked. Ruben and Kamen (*18*) concluded that the light decomposed primarily the H_2O but not the CO_2. Obviously this conclusion would have been

correct only if one could have brought forth the improbable argument that in light not the hydrate but only the anhydride of carbon dioxide reacted.

6) *"Hill" reactions.* If one suspends *Chlorella* cells in nitrate-nitric acid mixture, the cells develop O_2 for many hours when illuminated in the absence of CO_2, according to the equation

$$HNO_3 + H_2O = NH_3 + 2O_2,$$

a reaction that was discovered in 1920 (*19*). The mechanism of this reaction has been clarified as follows: the nitric acid oxidizes in a dark reaction the carbon of cell organic matter to carbon dioxide, and then, in light, splitting of the carbon dioxide into C and O_2 occurs, as in ordinary photosynthesis:

(Dark) $HNO_3 + 2C + H_2O = NH_3 + 2CO_2$
(Light) $2CO_2 = 2C + 2O_2$
(Balance) $HNO_3 + H_2O = NH_3 + 2O_2$.

In 1955 we found (*20*) that one can replace the nitric acid by ferricyanide:

(Dark) $4Fe^{3+} + C + 2H_2O = 4Fe^{2+} + 4H^+ + CO_2$
(Light) $CO_2 = C + O_2$
(Balance) $4Fe^{3+} + 2H_2O = 4Fe^{2+} + 4H^+ + O_2$.

Both reactions, with living *Chlorella,* appear in the balance as though water were decomposed by light, and as though the oxidizing agent acted only as a hydrogen acceptor, whereas in reality the light reaction is ordinary photosynthesis.

If, in the experiments with living *Chlorella,* one substitutes quinone for the nitric acid or the ferricyanide, CO_2 cannot participate in the development of O_2, since quinone completely inhibits the splitting of CO_2. Likewise, with green grana, CO_2 cannot participate in the development of O_2 since illuminated green grana are unable to reduce CO_2.

One must therefore either postulate two different mechanisms of photochemical O_2 development in green grana and in intact cells, which is improbable, or one must attempt to find a common explanation for the two phenomena: for water decomposition with intermediate photosynthesis, and for water decomposition without intermediate photosynthesis (*6*).

7) *The experiments of F. W. Allen.* In order to test whether photosynthesis without O_2 is possible, Allen (*21*), at the suggestion of James Franck, made use of the fact that the phosphorescence of many dyes is diminished by traces of O_2. A stream of nitrogen containing CO_2 was conducted over glowing copper, from there over water, then over a suspension of *Chlorella,* over liquid nitrogen, and finally over the dye acriflavin adsorbed on silica gel. Allen still found detectable photosynthesis at an O_2 pressure of 10^{-6} mm-Hg; whereas our manometry in closed vessels showed, without possibility of error, that below 10^{-1} mm-Hg photosynthesis in *Chlorella* is very small. What is the meaning of this discrepancy of five powers of 10?

The method of James Franck must be calibrated empirically—that is, each O_2 pressure yielding a given phosphorescence must be analytically determined. Furthermore, O_2 pressures of the order of magnitude of 10^{-6} mm-Hg must be produced, maintained, and analytically measured in a rapidly flowing gas. Anyone who is accustomed to performing experiments himself knows that this is an almost insoluble task. In any event, the analytical determination of traces of O_2 is the key aspect of the Franck method, and since Allen himself remained silent about this point, one must seek the mistake here. The calibration was obviously false by five powers of 10.

On the other hand, experiments of Hill and Whittingham (*22*) agree very well with our results. These workers added reduced hemoglobin to a suspension of *Chlorella* and determined the O_2-development upon illumination by optical measurements of the resulting oxyhemoglobin. They found that photosynthesis already began to fall off at an O_2 pressure of about 2 mm-Hg.

8) *The experiments of Allan Brown on "light-respiration."* When the light reaction and the dark reaction overlap in photosynthesis under normal conditions, two-thirds of the O_2 developing in the light will be reabsorbed so rapidly that one can think of the oxygen as oscillating between the free state and a binding with carbon. If the molecular O_2 provided the *Chlorella* is isotopically marked, whereas the CO_2 provided is not marked, one cannot expect that more marked O_2 will be consumed in light than in darkness, since in light, on spatial grounds, the unmarked O_2 photosynthetically produced within the cells will be consumed more rapidly than the externally available marked O_2 that must diffuse into the cells. Brown (*23*) found, in fact, that in light there was no increase in marked respiration, but often even a decrease of marked respiration; that is, not only the light respiration but also the dark respiration favored the unmarked oxygen produced within the cells photosynthetically from unmarked photolyte. This is a beautiful example of "isotopic discrimination."

The light respiration during illumination has also been the object of attempted measurement elsewhere, for example by Weigl, Warrington and Calvin (*24*), who illuminated green cells in marked CO_2 and expected that in light unmarked CO_2 would be given off in increasing quantity. They found, however, no increase in unmarked CO_2, quite in agreement with our equations, from which it follows that the light respiration must be marked when the CO_2 is marked.

In fact, the light respiration can only be measured as it was first discovered (*4*): when it is separated in time from O_2 development (*25, 26*).

References and Notes

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9. Here bolometry, introduced by the American physicist S. P. Langley, was developed and adapted to photochemistry by Lummer and Kurlbaum. It was Lummer's bolometer that played a decisive role in the discovery of light quanta. It was the bolometer of Lummer and Kurlbaum that was used to measure light energy in the experiments of Emil Warburg that laid the foundation of quantitative photochemistry. It is the same bolometer that has now solved the problem of the energetics of photosynthesis (F. Kurlbaum, *Wiedemann's Ann. Physik* 65, 746 (1898); O. Lummer and E. Pringsheim, *Verhandl. deut. physikal. Ges.* 1, 23 (1899); 2, 163 (1900); M. Planck, *ibid.* 2, 237 (1900); E. Warburg *et al., Ann. Physik* 40, 609 (1913); E. Warburg and C. Müller, *ibid.* 18, 245 (1916); E. Warburg, *Z. Elektrochem.* 27, 135 (1921).
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25. Reprints of this article may be obtained from the translators. A general review monograph, "Problems in Photosynthesis," by W. Bladergroen, will appear later in the year (Thomas, Springfield, Ill.).
26. *Note added in proof.* Further studies clarifying the mechanism of Hill reactions are in press: O. Warburg and G. Krippahl, "Hill-reactionen" and "Weiterentwicklung der manometrischen Methoden"; O. Warburg *et al.,* "Oxygenase in *Chlorella,*" *Z. Naturforsch.*