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ENERGY CAPTURE AND CONVERSION BY PHOTOSYNTHESIS

J. A. Bassham

February, 1962

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ENERGY CAPTURE AND CONVERSION BY PHOTOSYNTHESIS

J. A. Bassham

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Photosynthesis provides both the energy and the substance for the living world. Plants absorb light and convert it to chemical potential energy which drives all biological activity. When this chemical energy has been expended, it can be replenished only by photosynthesis. Without it, life would soon stop.

Thus, light from the sun is the primary source of energy for virtually all life on earth. An insignificant addition is the small amount of artificial light used by plants in houses and laboratories. Only the photosynthetic cells of plants and a few species of photosynthetic bacteria can employ light itself as a source of energy. These organisms convert light into forms of chemical potential useable by all living cells.

What is the nature of this unique process? In order to understand its basis, we must examine some of the fundamental chemical facts of life.

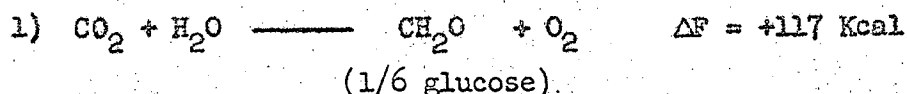
* The work described in this paper was sponsored by the United States Atomic Energy Commission.

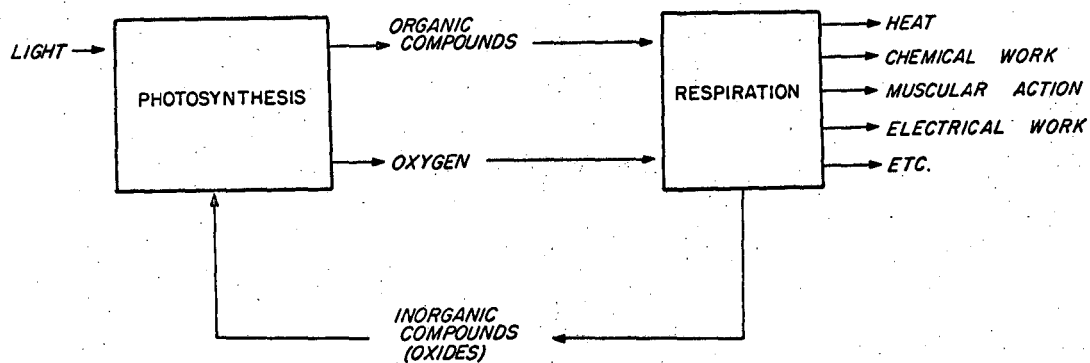
We live in an atmosphere which is about one fifth oxygen gas, O_2 . Oxygen gas has a powerful tendency to react with the materials of life - the organic compounds. When this happens, four of the most important elements of organic material are converted to oxides. These elements are hydrogen, carbon, nitrogen and sulfur. Their most common oxides are water, carbon dioxide, sulfate and nitrate, all a part of the world of inorganic compounds.

The reaction of oxygen with organic compounds to form oxides releases large amounts of energy. For example, wood or coal burns in the oxygen of the air, forming water and carbon dioxide and releasing much heat. By means of more controlled reactions all aerobic (oxygen-requiring) organisms permit oxygen to react with organic materials as a source of energy for life processes. This biological combustion is respiration.

Photosynthesis is almost the opposite of respiration. Green plants absorb light and use its energy to separate some of the oxygen from its oxides and to form organic compounds from the remaining atoms. The relations between photosynthesis and respiration in the overall economy of the biosphere (the world of living organisms) is shown in Fig. 1. This diagram illustrates the cyclical path of matter and the non-cyclic flow of energy through these systems.

The formation of organic compounds by photosynthesis and the storage of chemical potential energy can be expressed by equations which tell us the overall result. For example, the formation of one sixth mole of glucose, under physiological conditions, from water and carbon dioxide, can be expressed by the equation:





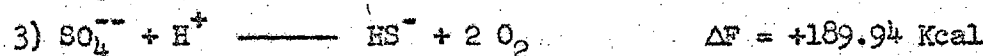
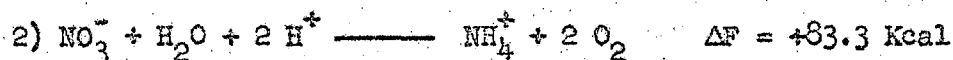
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Fig. 1. Relationship of photosynthesis to respiration in the biosphere.

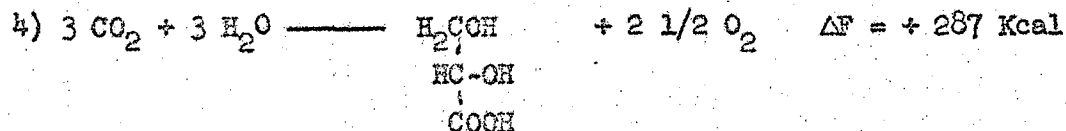
The +117 Kcal free energy change in this reaction means that as the equation is written, 117 Kcal of useful work must be supplied for each mole of carbon dioxide reduced to make the reaction go. This energy comes from the light absorbed by the plant pigments.

The reverse reaction (right to left) is respiration. A respiring cell burning one sixth mole of glucose with oxygen, can theoretically obtain up to 117 Kcal of useful work for cellular functions such as chemical synthesis or osmosis. A multicellular organism can use some of this work for such energy requirements as muscular activity (mechanical energy), nerve transmission (electrical), or, if it is a firefly, illumination (electromagnetic).

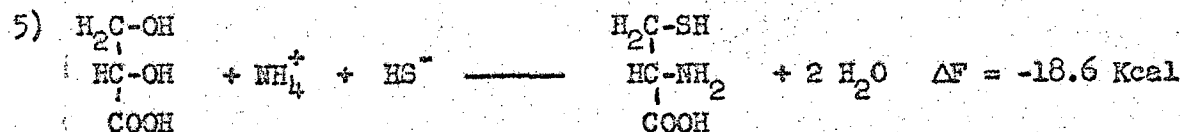
Not only carbon dioxide is reduced by photosynthesis, but other oxides, particularly nitrate and sulfate, as well:



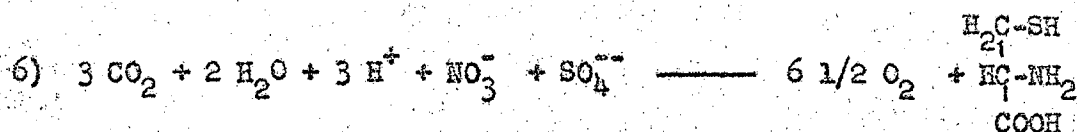
Some of the carbon dioxide is not reduced to carbohydrate, but instead is converted to other metabolic intermediates such as glyceric acid:



Then this carbon compound metabolite plus the reduced nitrogen and sulfur are used to make an amino acid such as cysteine:



By adding equations 2-5 we find the total reaction from the inorganic oxides to cysteine and oxygen:



$$\Delta F = +541 \text{ Kcal}$$

This exercise illustrates some rather important points about photosynthesis, and in fact about all biosynthesis:

1. By far the greater part of the energy required for synthesis of biological organic compounds is used to remove electrons from oxygen atoms, producing oxygen gas and at the same time reducing the other elements to less oxygenated compounds.

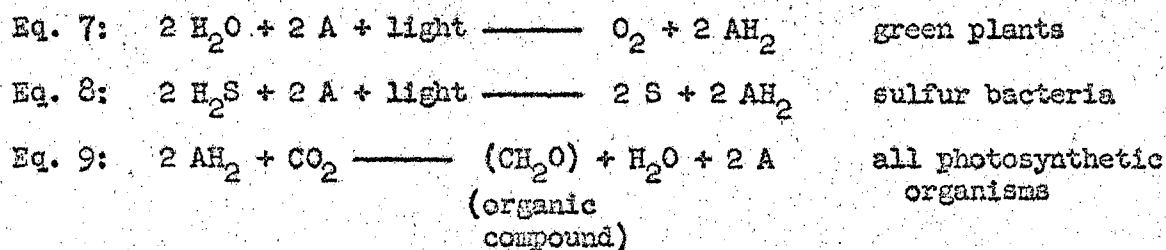
2. Once the other elements (carbon, nitrogen and sulfur) are reduced, subsequent reactions leading to larger and more complex molecules require relatively small amounts of additional energy, unless further reduction is required. Of course, when further reduction occurs, as in the formation of fats from carbohydrates, more electrons must be removed from the oxygen in oxides (in this case water) and additional large amounts of energy must be used.

The amount of energy per mole of electrons transferred from water to carbon in the formation of carbohydrates and other compounds with about the same ratio of carbon to oxygen (1 to 1) is 25-30 Kcal (1.1 to 1.3 volts). For more reduced compounds such as fats the energy required per mole of electrons transferred is less - about 20 to 25 Kcal (0.9 to 1.1 volts).

In order to discuss the mechanism of this transfer of electrons from oxygen to carbon and other atoms, we must go back some time in the development of our present knowledge of the chemistry of photosynthesis.

Over 30 years ago, Cornelius van Niel¹ pointed out that certain photosynthetic bacteria use light to produce organic compounds in a manner similar to green plants but with one important difference. These bacteria

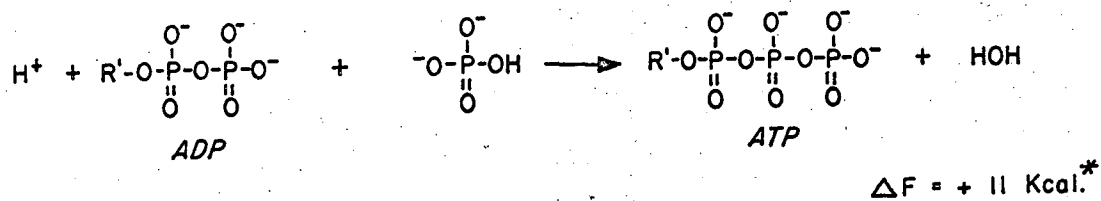
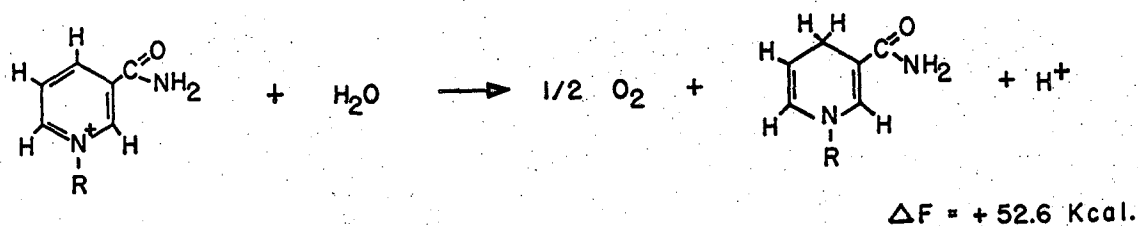
use hydrogen sulfide, H_2S , and liberate elemental sulfur instead of oxygen gas. Van Niel proposed that these two processes differed mainly in their choice of a source of hydrogen atoms. Thus, plants split water to give some form of "bound hydrogen", AH_2 , and oxygen gas (Equation 7), while sulfur bacteria split hydrogen sulfide to give AH_2 and sulfur (Equation 8). Both the green plants and sulfur bacteria then use the "bound hydrogen" to convert carbon dioxide to organic compounds (Equation 9).



Note that for each two molecules of water originally split, one molecule of water is reformed, when carbon dioxide is reduced to carbohydrate. Thus all the oxygen evolved in photosynthesis comes instantaneously from water, even though in the overall reaction of photosynthesis about half of the oxygen liberated ultimately comes from CO_2 , NO_3^- , SO_4^{--} , etc.

Compounds which carry electrons or hydrogen atoms in biological systems are but one of many types of cofactors, or coenzymes. Coenzymes are molecules which work with enzymes to bring about reactions. Enzymes are biological catalysts - substances which cause specific biochemical reactions to proceed at great rates, but which are not permanently changed themselves.

The coenzyme which carries electrons in photosynthesis is triphosphopyridine nucleotide (TPN^+). It is a two-electron carrier, and when it receives the electrons which come ultimately from water, it undergoes the reaction shown in Eq. 10, from left to right. The reduced form ($TPNH$) thus corresponds to van Niel's AH_2 (Eqs. 7-9).



* ASSUMING: $[\text{HPO}_3^{2-}] = 10^{-3} \text{ M}$
 $[\text{H}^+] = 10^{-7} \text{ M}$
 and $[\text{ADP}] = [\text{ATP}]$

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Equations 10 and 11.

You will note that the energy required per mole of electrons transferred from oxygen to this two-electron carrier is 26 Kcal (1.1 volts) - just about the average energy needed to bring about the transfer of an electron from the oxygen atom of water to a carbon atom in forming an organic compound. Thus most of the energy needed to make the organic products of photosynthesis is stored temporarily in this reduced cofactor. However, somewhat more energy is required to make such compounds as carbohydrates, and moreover, energy transfer in biological systems, although remarkably efficient, can never be 100% efficient. For these reasons, and others, an additional carrier of useable chemical energy is required for the photosynthesis of carbon compounds from CO_2 .

This energy carrier is another coenzyme which is of great importance in all biochemical systems; adenosine triphosphate (ATP)². Actually ATP is an acid anhydride, and it owes its energy storing ability to the fact that acid anhydrides upon hydrolysis (reverse of Eq. 11) give up free energy.

During photosynthesis, some of the energy absorbed by the plant is somehow used to form this anhydride from the two acids ADP and inorganic phosphate which exist in their ionized form in physiological pH (about 7). Unlike most acid anhydrides, ATP is moderately stable in aqueous solution. Thus its energy can be released or used by enzymes which catalyze specific reactions.

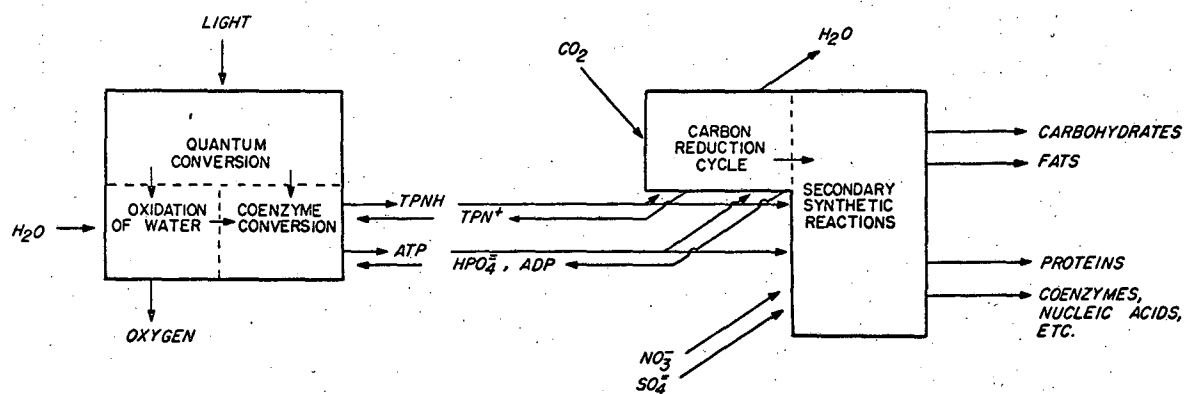
For the reduction of carbon dioxide in photosynthesis, there are two such reactions of particular importance. In each of these reactions the terminal group of ATP is transferred to an organic molecule where it serves to activate the molecule towards a subsequent biochemical reaction that would not otherwise take place.

In Fig. 2 we see the relation between the reactions which convert the cofactors to their energetic and reduced forms, and the reactions which utilize the cofactors for the reduction of carbon dioxide and other oxides and the synthesis of organic compounds. Following the absorption of light by the plant pigments, the energy of the excited pigment is converted to some form of chemical potential. This chemical potential in turn is used to split water by removing electrons from the oxygen atom of water and transferring them to TPN^+ to make TPNH . At the same time, some of the light energy is utilized in converting adenosine diphosphate and inorganic phosphate to the acid anhydride ATP.

These two cofactors then are used in the primary carbon reduction cycle to make organic metabolites. These organic metabolites in turn are used in the synthesis of larger molecules such as carbohydrates, fats and proteins. The synthesis of proteins of course also requires the reduction and incorporation of sulfate and nitrate, as does the synthesis of some cofactors and nucleic acid bases.

For the purposes of discussion, these two sets of reactions may be considered separately. We shall turn our attention first to the synthetic reactions, about which we know more. The basis of these synthetic reactions is the carbon reduction cycle (Calvin Cycle) mapped by Professor Calvin and associates^{3,4}. This cycle is shown in simplified form in Fig. 3.

Energy input into this cycle may be considered as starting with the reaction of ATP with ribulose monophosphate to form ribulose diphosphate.



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Fig. 2. Relationship of early stages to carbon reduction in photosynthesis.

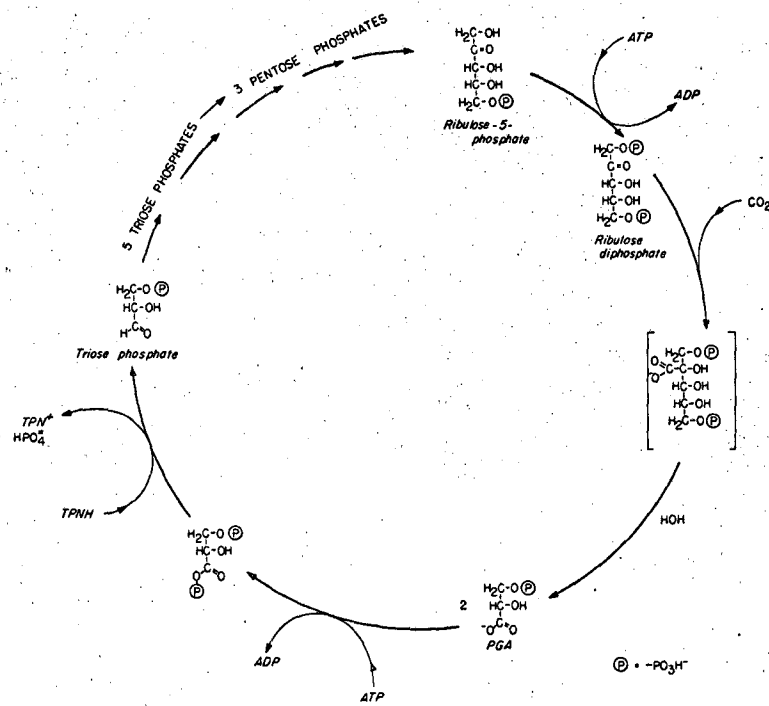


Fig. 3. Simplified carbon reduction cycle.

This addition of a second phosphate group to the sugar molecule makes it sufficiently reactive to undergo a carboxylation reaction in the presence of the enzyme carboxydismutase. In fact, ribulose diphosphate is the only sugar phosphate found thus far which is capable of undergoing such a reaction. In the carboxylation reaction, carbon dioxide is incorporated into the carboxyl group of an unstable six carbon compound, shown in brackets, which then splits to two three carbon compounds. As shown here, these two carbon fragments are phosphoglyceric acid, PGA.

Before the carboxyl group of PGA can be reduced, it must first be activated in a reaction with adenosine triphosphate. The terminal phosphate group of adenosine triphosphate is transferred to the carboxyl group, making a new acid anhydride. This acid anhydride, is much more reactive than the free acid and undergoes reduction by TPNH. The products of the reaction are inorganic phosphate, TPN^+ and 3-phosphoglyceraldehyde, a three carbon sugar phosphate (triose phosphate).

A number of reactions then occur which result in the conversion of five molecules of triose phosphate to three molecules of ribulose monophosphate. There is no input of energy in the form of energetic cofactors to any of these reactions. The pentose monophosphate is of the same reduction level as the triose phosphate, and the reactions leading from triose phosphate to pentose phosphate are all downhill reactions in terms of energy.

Each of the three molecules of pentose phosphate are converted by ATP to pentose diphosphate. These pentose diphosphates then react with three molecules of carbon dioxide. Finally, six molecules of PGA are formed. Thus, for each complete turn of the cycle there is incorporation of three molecules of carbon dioxide and the production of one extra three carbon organic compound, such as triose phosphate.

The bill for this carbon reduction is six molecules of TPNH and nine molecules of ATP per complete cycle revolution. Put another way, two molecules of TPNH and three molecules of ATP are required for each molecule of carbon dioxide reduced to the level of carbohydrate.

We have already said (Eq. 1) that the energy stored in the reduction of carbon dioxide to the level of carbohydrate is 117 Kcal, when water is split as a source of electrons. The coenzyme energy bill can be obtained by multiplying Eq. 10 (TPNH formation) by two, giving 105.2 Kcal and Eq. 11 (ATP production) by three, giving 33 Kcal. The total comes to 138 Kcal worth of coenzyme energy expended. We can therefore calculate that the efficiency of the carbon reduction cycle as just formulated is 117 over 138, or 85% efficient.

This is a remarkable efficiency, particularly if we consider the cycle in all its details, as shown in Fig. 4. There we see some 15 or more biochemical steps, all of which are proceeding with a net rate in the forward direction under steady state conditions of photosynthesis. The difference between 138 Kcal expended and 117 Kcal stored, or 21, represents the driving force which makes these reactions proceed in the forward direction.

Let us examine an isolated step in these biochemical pathways. Consider step

$A + B \xrightleftharpoons[k_2]{k_1} C + D$, at steady state, where f = forward reaction, b = back reaction.

$$\frac{f}{b} = \frac{k_1 [A] [B]}{k_2 [C] [D]} \quad (\text{whether reaction rates are first order or not})$$

$$\Delta F^S = \Delta F^O - RT \ln \frac{[A] [B]}{[C] [D]} = -RT \ln \frac{k_1 [A] [B]}{k_2 [C] [D]} = \Delta F^S = -RT \ln \frac{f}{b}. \quad \text{The free energy}$$

change at steady state is thus related to the forward and backward rates. Note that ΔF^S represents the free energy change under steady state conditions. In other words, the activities have been corrected for the concentrations of metabolites actually existing during the steady state conditions⁴. This means that the reactants and the products along the biochemical pathway have been so adjusted that the free energy of every step is negative. The magnitude of this negative free energy change per step is a reflection of the difficulty of that particular

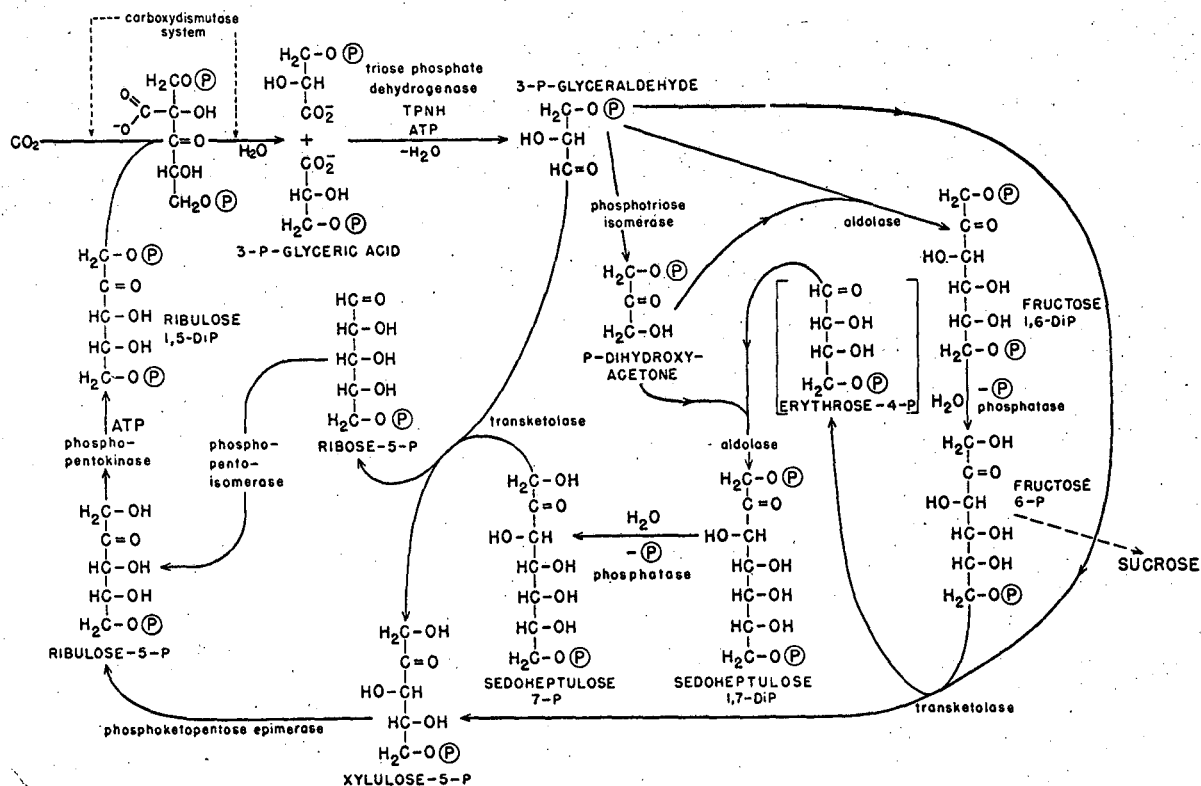


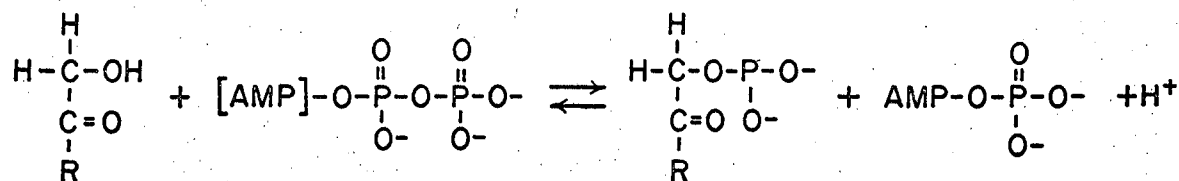
Figure 4. Carbon reduction cycle.

step, or a sort of chemical friction which has to be overcome by the potential as represented by the negative free energy.

We can illustrate this more specifically by viewing the actual steps in the carbon reduction cycle. The first of these is the phosphorylation reaction, in which ATP donates its terminal phosphate group to ribulose monophosphate. At steady state conditions the free energy of this reaction is estimated at about -3 Kcal. Thus, of the 11 Kcal available from the ATP molecule, about 8 are stored in the sugar phosphate to make it more reactive and three are used to drive the reaction in the forward direction.

I

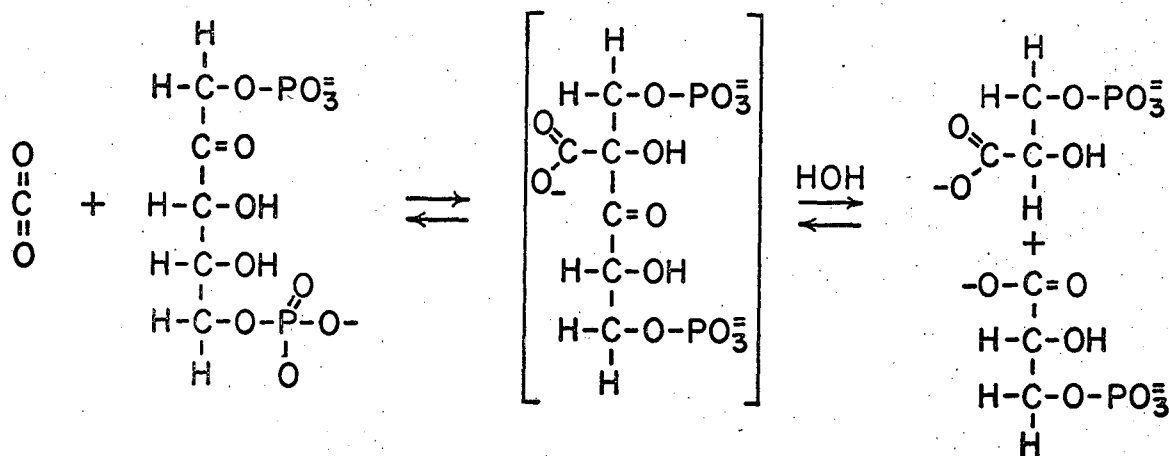
Phosphorylation Reaction



$$\Delta F^S = -3.1 \text{ Kcal}$$

Next comes the carboxylation reaction in which CO_2 adds to the five carbon sugar diphosphate, the product is hydrolytically split to make two molecules of PGA. The free energy change of this reaction is estimated at at least -7 Kcal. This relatively large free energy change under steady state conditions follows from the fact that the reaction is a rather complex one involving several steps and consequently a rather high degree of chemical friction.

II Carboxylation Reaction

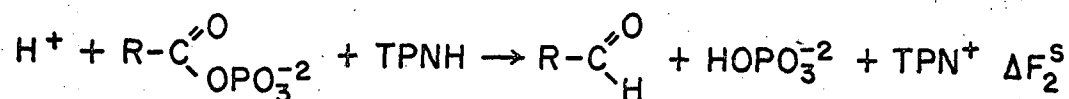
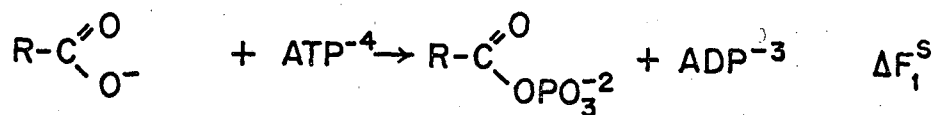


$$\Delta F^S = -7.3 \text{ Kcal}$$

The next step is the reduction reaction, and the first step in the reduction is the phosphorylation by ATP of the carboxyl group of PGA. The intermediate in this case has not been measured, but it is reduced by TPNH, giving the triose phosphate, which is an aldehyde, inorganic phosphate, and oxidized TPN,¹ and the total free energy change for these two steps is -2.8 Kcal. However, this value is based upon certain assumptions as to the relative levels of the coenzymes involved.

III

Reduction Reaction



$$\Delta F^S = \Delta F_1^S + \Delta F_2^S = -2.8 \text{ Kcal}$$

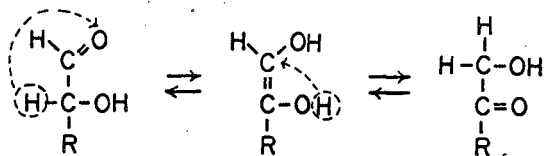
$$\text{when } [\text{HPO}_4^{-2}] = 10^{-3} \text{ M}, \quad \frac{\text{ATP}}{\text{ADP}} = 1, \quad \frac{\text{TPNH}}{\text{TPN}} = 1$$

A number of steps are involved in going from the triose phosphate to the pentose phosphate. We will not consider each of these in detail, but I will discuss the four kinds of reaction. The first of these is a kind of epimerization reaction, in which only the movement of a hydrogen atom is involved. This is a rather simple reaction and quite predictably, its free energy change under steady state conditions is small. The second type is a condensation reaction, in which there is formed a new carbon-carbon bond while at the same time the hydrogen atoms move. In this case the free energy change is somewhat greater, indicating a higher degree of chemical friction.

IV

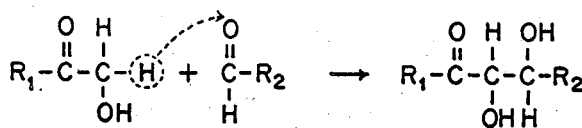
Sugar Rearrangement Reactions

A. Epimerization



$$\Delta F^S = -0.1, -0.1, -1.6 \text{ Kcal}$$

B. Condensation



$$\Delta F^S = -3.8, -3.3 \text{ Kcal}$$

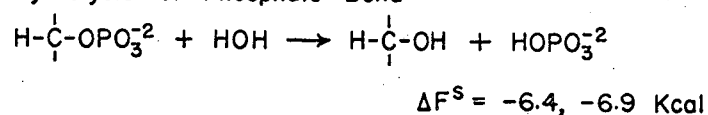
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Also involved are hydrolyses of phosphate bonds, in the case of fructose diphosphate and sedoheptulose diphosphate. These hydrolyses involve rather large negative free energy changes. This stems not so much from the reaction being a complicated one, but rather from the fact that under steady state conditions there is no good way to utilize the energy liberated in this particular biochemical sequence. As the result, the concentrations and the activities of the diphosphates drop to very low levels during steady state photosynthesis, and this introduces an entropy term when these reactants with very small activities have to undergo hydrolysis. Consequently, the relatively large free energy change occurs.

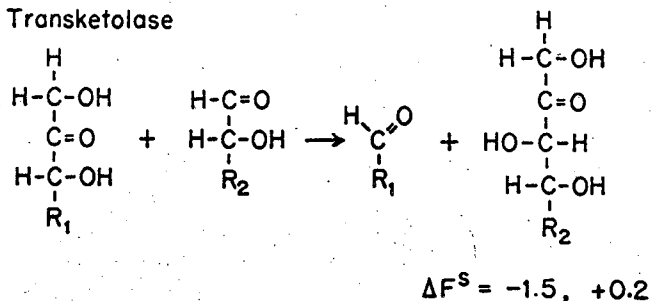
IV

Sugar Rearrangement Reactions

C. Hydrolysis of Phosphate Bond



D. Transketolase



The final type of reaction involved is a transketolase reaction, in which the two top carbon atoms of one sugar are transferred to another sugar, causing a dismutation in the chain length of the two sugars. This would seem to be a fairly complex reaction, but actually its mechanism, not shown here, which involves addition of the two carbon fragment first to thiamine pyrophosphate, is in the nature of an equilibrium reaction and its free energy change is again rather small.

In summary, the negative free energy expenditure may be thought of as providing a driving force to make reactions go at a rapid net forward rate. The distribution of free energy among the various reactions depends upon the complexity or chemical friction of the particular step involved.

Now let us turn our attention to the central problem of photosynthesis, the conversion of light or electromagnetic energy into chemical potential and the consequent production of cofactors in their reduced and more energetic forms. First of all, how much energy is available in the light utilized by the plant for photosynthesis? This question consists of two parts: 1) What is the energy of the light photons used by the plant? and, 2) How many photons are required for a given amount of photosynthesis?

The principle pigments in most plants are two forms of chlorophyll, chlorophyll a and chlorophyll b. These pigments have their main absorption in the blue and in the red. The other most common pigment found in higher plants and algae are carotenoids. The sum of the absorption by these pigments and other accessory pigments is such that the plant absorbs throughout the visible, but with somewhat less absorption in the green and yellow. The result is that most plants appear green to our eye, due to the relatively greater reflection of the green and yellow wavelengths.

It has been shown that light of any wavelength from 4000 to 6800 absorbed by the various plant pigments can be effective for photosynthesis⁵, although the efficiency with which light absorbed by the carotenoids is used is sometimes less than the efficiency with which chlorophyll-absorbed light is used. In any event, it is generally accepted that whatever the wavelength of the light absorbed, the resulting excited states of the pigment are energetically degraded to the level of the excited state of the pigment if it were to absorb in the longer wavelengths. Specifically, it appears now that excited states of pigments corresponding to the absorption of about 670 mμ and 700 mμ are the states actually involved in the energy conversion during photosynthesis. Thus, we must assume that there is available for the subsequent conversion to chemical potential only that amount of energy corresponding to light of the wavelengths 670 and 700 mμ. The additional energy corresponding to light of shorter wavelengths is presumed to be lost in the conversion of one excited state to another.

If we apply the equation $E = h \nu$, where h is Planck's constant and ν is the frequency of light, and use suitable factors to convert from ergs to calories, and if we also multiply by Avogadro's number to go from an individual photon to a mole of photons (an einstein), we find that light at 670 mμ has an energy of 42.7 Kcal per einstein while light at 700 mμ has an energy of 40.9 Kcal per einstein.

We need also to know how many photons are actually used in the reduction of one molecule of carbon dioxide and the evolution of one molecule of oxygen during photosynthesis. You will recall that in the formation of carbohydrate there is a transfer of four electrons from oxygen to carbon for each molecule of oxygen gas evolved from water. Were photosynthesis a simple photochemical reaction in which one electron was transferred for each quantum, it is obvious

that the reduction of one CO_2 molecule would be a four quantum process. Thus, the energy available from the light would be four times 40, or about 160 Kcal per mole of CO_2 , more than enough to bring about a net free energy storage of 117 Kcal in the formation of carbohydrate from carbon dioxide and water. In fact, four einsteins would more than pay the total coenzyme energy bill per mole of carbon dioxide reduced, which was two moles of TPNH and three moles of ATP, or 137 Kcal in all.

The quantum requirement of photosynthesis has been studied for many years by a great number of investigators. Many years ago, Warburg⁶ found quantum requirements of about four and he continues to find low requirements, sometimes as low as three⁷. The vast majority of other investigators studying this problem have found much higher quantum requirements for photosynthesis, and there are at present three schools of thought. One group believes the quantum requirement to be eight⁵, that is, two light quanta per electron transferred, and one group, principally Warburg and coworkers, believe the quantum requirement to be four or less. There is another group^{8,9} who feel that the quantum requirement is somewhere in between, perhaps six or seven. We are in the latter camp, having measured in our own laboratories quantum requirements of about seven, which we believe to be sufficiently accurate to enable us to conclude that the quantum requirement is definitely less than eight¹⁰.

Since the quantum requirement measurements have been generally inconclusive, most workers have abandoned them in favor of trying to learn more about the specific mechanisms of the reactions, which will in time tell us exactly what the quantum requirements of photosynthesis are. It would take volumes to describe the work, conclusions and hypotheses in this field, and

Richard E. Smith

I shall confine myself here to presenting a scheme which I believe represents the latest hypothesis for the conversion of light energy and the transfer of electrons from water to cofactors with the consequent formation of ATP and TPNH.

What are the key substances involved in this light conversion and electron transfer? First there appear to be two pigment systems, each of which has a role in capturing light energy and holding that energy in an excited state of the molecule until it can be converted to chemical potential. The two pigment requirement is a rather recent development, stemming from the work of Emerson¹¹ and elaborated by the work of Meyers^{12,13}, French¹⁴ and others. Briefly, these studies show that light of around 700 mμ which is absorbed by the plant is not effective by itself in producing oxygen evolution and photosynthesis. However, if light of 670 mμ is also added, then photosynthesis is produced not only by the added light but by the 700 mμ light. In other words, the two different kinds of light are acting together to produce more photosynthesis than the sum of the two acting independently. Different pairs of wavelengths than the pair just described are required for certain specialized photosynthetic organisms.

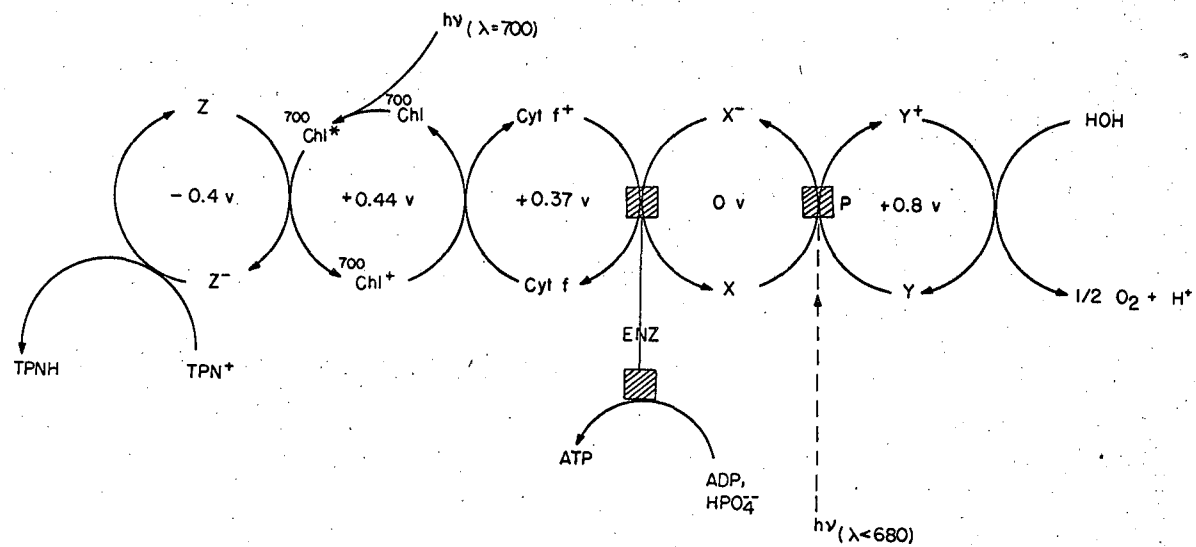
Besides the pigment systems, it appears that a cytochrome pigment¹⁵, particularly cytochrome f, is involved in the primary act of photosynthesis. It was found that when chlorophyll a was illuminated there was an immediate oxidation of cytochrome f¹⁶. Chance demonstrated that this oxidation of cytochrome by chlorophyll could occur even at liquid nitrogen temperatures¹⁷. This meant that the cytochrome oxidation was a solid state reaction - one which did not require the movement of atomic nuclei. Evidence for the formation of unpaired electrons and free radicals at liquid nitrogen temperatures had previously been obtained from electron spin resonance studies by Calvin and coworkers¹⁸.

Other substances which appear to be implicated in electron transport from water to TPN^+ included plastoquinone¹⁹, perhaps other quinones, such as vitamin K²⁰, and manganese²¹.

Using plants in which the two pigment systems were sufficiently different to permit them to be illuminated separately, Duysens¹⁶ found that illumination of chlorophyll a caused the oxidation of cytochrome, while illumination of the accessory pigment caused the reduction of the cytochrome. Furthermore, certain inhibitors of oxygen evolution in photosynthesis were found by Duysens to inhibit the reduction of the cytochrome by this accessory pigment.

The conclusion to be drawn from the facts just presented is that in the plant just described, light absorbed by the accessory pigment results in the movement of electrons from water to the reduction of cytochrome f while light absorbed by chlorophyll a results in the movement of electrons from the cytochrome f to the final electron acceptor. The latter substance in turn would reduce TPN to TPNH . These conclusions find further support in the work of Witt²², who has applied flashes of bright light of various wavelengths to photosynthetic organisms and has studied the transient changes in the absorption spectra at various wavelengths, caused by these flashes of light. He has found a number of different transient changes with various time constants for their formation and decay. By many careful experiments he has been able to assign these changes to specific steps in the electron transport chain.

Witt has added to his systems substances capable of carrying out oxidation and reduction at various points on the chain and knowing oxidation reduction potentials of these added substances, he has assigned oxidation reduction potentials to various cofactors involved in photosynthetic electron transport on the basis of their reaction with added substances. As a result of his studies and his assignments he has postulated the scheme shown in Fig. 5 .



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Fig. 5. Electron transport in photosynthesis (after Witt).

The essential features of this scheme are as follows: Beginning at the left hand side, a photon of light, $h\nu$, is absorbed by the chlorophyll pigment and causes the transfer of an electron from chlorophyll to some acceptor, Z. According to Witt, chlorophyll⁺ has an oxidation potential of +.44 volts, and Z an oxidation potential of -.4 volts, so that the light has accomplished the transfer of an electron against a potential of .84 volts, about 19.4 Kcal per mole, and well within the 40.9 Kcal available from an einstein of red light. In its reduced form, Z is a sufficiently strong reducing agent to reduce TPN^+ to TPNH.

In the meantime, the positively charged chlorophyll a, which in reality may be an electron hole in an array of chlorophyll molecules, as suggested by Calvin¹⁸, reacts with the cytochrome in a nonenzymic, dark reaction, to make the reduced form of the cytochrome.

The reduced form of the cytochrome then undergoes an enzymic reaction with some other coenzyme with about 0 volts oxidation potential. The energy liberated in this process is coupled with the reaction leading to the formation of ATP from ADP and inorganic phosphate. The amount of energy liberated for one electron would be 19.4 Kcal, more than enough to make an ATP molecule. However, such reactions are usually considered to require two electrons transferred per ATP formed, in which case there would be some 38 or 39 Kcal, enough to make three molecules of ATP if the reaction went in several steps.

The second light reaction, one which in some plants is accomplished by accessory pigments, causes the transfer of an electron from a hypothetical substance Y to X, forming in the process a very strong oxidizing agent, Y^+ , with a potential of +0.8 volts, enough to strip an electron from the oxygen of

water and form molecular oxygen. Presumably the reaction between Y and X^+ is also a one electron transfer, which would result in the storage of some 18.4 Kcal energy from the 42 Kcal available in 670 mμ light.

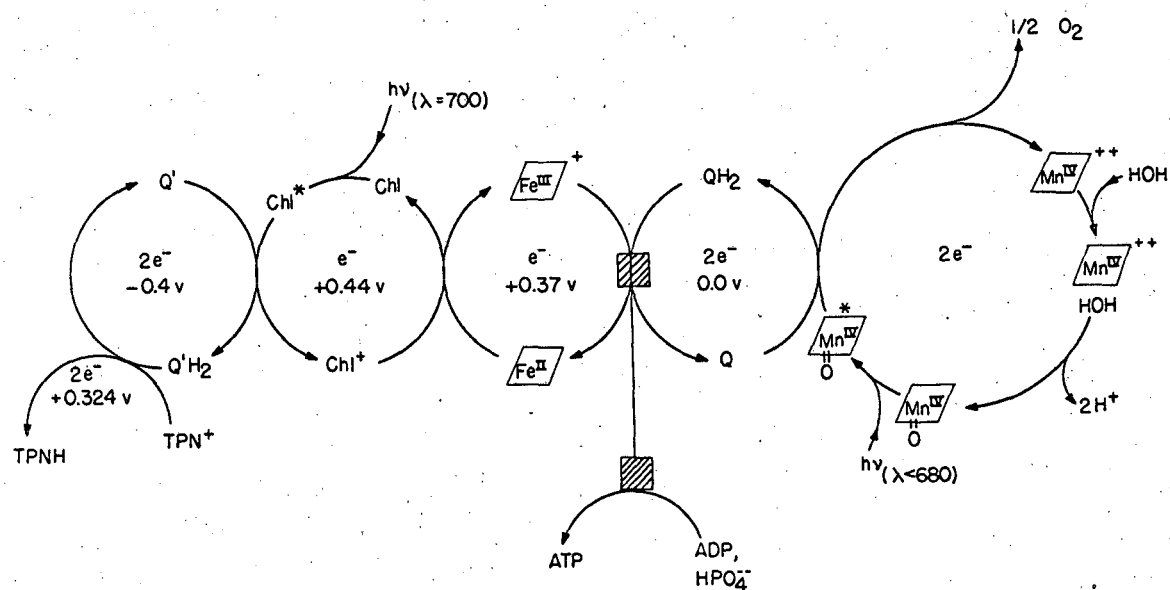
As just postulated, there would be two photons required for the transfer of each electron from the oxygen of water to TPN^+ to make TPNH, and consequently, since four electrons must be transferred for each carbon dioxide reduced, this would be an eight quantum requirement process. Since I believe the quantum requirement may be less than 8, I am led to postulate a modified scheme in which one of the photochemical steps transfers not just one electron, but two. If we allow the other photochemical step to transfer just one electron, we end up with a quantum requirement of 1.5 per electron, or six quanta per carbon dioxide molecule reduced.

At the same time, in order to suggest roles for the remaining substances, which are believed to be involved in electron transport, I have put them into my very hypothetical scheme, which is shown in Fig. 6.

Now this scheme is certainly the very sketchiest kind of hypothesis and the only thing certain about it is that it will be changed as soon as more information is known about the primary steps in photosynthesis. However, I believe it does illustrate the kind of system which is probably involved in electron transport and cofactor formation.

In the first step we have the 700 mμ light being used to excite chlorophyll a to an excited electronic state. Bear in mind that any light of shorter wavelength may have been absorbed either by chlorophyll a or b, or some accessory pigment and transferred to the particular chlorophyll a molecule in which the energy conversion is to occur.

For the purpose of capturing light quanta of all wavelengths there are



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Fig. 6. Electron transport in photosynthesis (modified version).

probably a great many more molecules of pigment than there are of those pigment molecules involved in the actual energy conversion. Park²³ has found a unit within the structure of the chloroplast which he calls the quantasome. This unit, which has been revealed by electron microscope pictures, appears to be an ultimate unit for the splitting of water and transfer of electrons from water to the reducing agent. It is about 100 Å by 200 Å- an oblate spheroid. We believe that chlorophyll molecules, about 200 to 300 of them, are somehow arrayed on the surface of this particle, the main body of which is protein. Sauer and Calvin²⁴ have evidence that of these chlorophyll molecules, perhaps twenty, are oriented in a regular array and they believe that this array of chlorophyll molecules is at a particular locus on the quantasome. Light absorbed by any of the pigment molecules of the quantasome travels by exciton migration or resonance transfer from one molecule to another until it reaches this particular locus of arrayed chlorophyll molecules. Then charge separation takes place, with an electron being transferred from a chlorophyll molecule to the electron acceptor, which I have shown as $Q'H_2$ in my scheme. The electron hole or positive charge resulting from this charge separation then migrates through the array of ordered chlorophyll molecules to another site at which it extracts an electron from cytochrome f, causing the oxidation of the cytochrome. The purpose of the array of chlorophyll molecules is to separate physically the electron acceptor and the electron donor. The potential between the acceptor and the donor are such that they would react with each other if they were in close contact. Once the electron acceptor is reduced, it then can reduce TPN to TPNH.

Then, just as in Witt's scheme, I have the oxidized cytochrome reacting to oxidize another cofactor which I have labeled QH_2 . This reaction may involve

the coupling of energy into the formation of ATP from ADP and inorganic phosphate. Since coenzyme Q, or ubiquinone, is thought to play a role in ATP formation during respiratory electron transport, we have assigned plastoquinone, a similar compound, the role of ATP formation and electron transport during photosynthesis. Its oxidation potential, moreover, appears to be about the one required for its position in this scheme.

The 670 mμ light reaction I visualize as an excitation of a manganese atom bound in a heme pigment in much the same way that iron is bound in heme in the cytochrome and magnesium is bound in chlorophyll. The excited manganese heme compound then undergoes a reaction in which two electrons are transferred from a double bond between manganese and oxygen to the plastoquinone, returning it to its reduced form. The remaining oxygen manganese bond is broken and eventually oxygen gas is evolved. I have not indicated any detailed mechanism for this oxygen evolution in the scheme. Conceivably carotenoid compounds, especially such oxygen carrying ones as luteine and violaxanthine may play a role in this process. The Mn^{IV} then reacts with a water molecule and forms once again a manganese oxygen double bond, liberating hydrogen atoms. Now this cycle, in which manganese in a heme pigment plays a key role, is the most hypothetical of all, and will almost certainly prove to be incorrect as formulated here. What I do wish to suggest is that manganese is in some way necessary for the photochemical transfer of two electrons per quantum of water to some cofactor with about the oxidation potential of plastoquinone. If this happens, some 37 Kcal of the 42.8 Kcal available in a 670 mμ einstein would be used.

For the formation of three moles of ATP and two moles of TPNH, the efficiency would be $137 / (2 \times 42.8 + 4 \times 40.9) = 56\%$, for the formation of

1/6 glucose and O_2 , from H_2O and CO_2 , the efficiency would be 47%.

Now I will summarize the capture and conversion of energy by photosynthetic plants:

Electromagnetic energy with wavelengths from 400 mμ to 700 mμ is absorbed by pigment molecules - chlorophyll a and b, and accessory pigments - which are probably distributed over the surface of a large protein molecule. The resulting excited state of the pigments is transferred from one pigment to another, until it reaches an organized array of chlorophyll molecules. There the energy of the excited state is converted by an essentially solid state process to a charge separation between an electron and an electron hole. The electron eventually reduces an enzymic cofactor, TPN^+ , making $TPNH$. The hole migrates through the pigment array, until it can oxidize a cytochrome molecule.

The oxidized cytochromes oxidize another coenzyme, and perhaps in the process produce ATP, this step being an enzymic reaction. The oxidized coenzyme must in turn get electrons from water, and since this reaction requires a large input of energy it must also use light absorbed by the pigment systems. This oxidation of water somehow requires manganese, perhaps in the form of a pigment. Also the water splitting reaction appears to utilize a different pigment system, and cannot be effected by absorption of light of wavelengths longer than 670 mμ.

The transport of four electrons (liberating one O_2 molecule) requires six or eight photons, depending on the assumptions made, and produces two molecules of $TPNH$ and two or more molecules of ATP. The overall efficiency for the production of these cofactors would not be much more than 50%, depending on the assumptions and based on the utilization of red (670 and 700 mμ) light. With shorter wavelengths the energy efficiency decreases in proportion to wavelength.

The coenzymes - three molecules of ATP and two of TPNH - are used with remarkable efficiency (perhaps 85%) to reduce CO_2 to carbohydrates and other products. ATP activates metabolites by increasing their energy content and making them more reactive towards carboxylation and reduction. TPNH, which carries the larger part of the chemical potential from the light reactions, reduces a carboxyl carbon to an aldehyde carbon. The energy of the coenzymes not stored as chemical potential in the final products, provides the driving force to make a complex series of reactions proceed at a rapid rate in the forward direction.

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