[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

Photosynthesis and Phosphorylation

BY S. RUBEN

There is little doubt that photosynthesis consists of a complicated sequence of thermal and photochemical reactions. It seems profitable to divide the mechanism of photosynthesis into four more or less distinct processes: (1) photochemical reactions involving chlorophyll in which radiant energy is used to produce oxidizing and reducing agents, the thermal (dark) (2) fixation of carbon dioxide as the carboxyl group of an organic compound, (3) the reduction of this substance and (4) production of molecular oxygen. This formulation is similar to that of van Niel and his publications should be consulted for support.[1] In this communication a model for the dark fixation and reduction of carbon dioxide is briefly presented.

Dark Fixation of Carbon Dioxide.—Evidence has been obtained[2] which indicates the initial reaction of carbon dioxide in photosynthesis to be a reversible thermal carboxylation reaction. This process has been represented by equation (1), which is to be considered as a type reaction since RH may not be unique in composition.

$$\mathrm{RH} + \mathrm{CO_2} \underset{}{\overset{\text{Enzyme}}{\rightleftarrows}} \mathrm{RCOOH} \qquad (1)$$

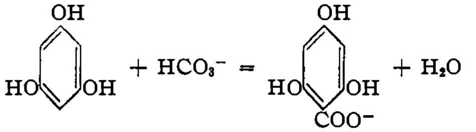
In the green alga *Chlorella pyrenoidosa*, the equilibrium state appears to be mainly to the right[3] (ΔF approx. -2 kcal.).[4] However, for carboxylation equilibria such as (1) it seems that the free energy change is very unfavorable. A compilation of the values of ΔF from the available published literature[5] for carboxylation reactions is presented in Table I.

TABLE I

	Reaction	ΔF_{298}
1.	CH_4 (g) + CO_2 (g) = CH_3CO_2H (aq.)	+10.4
2.	C_2H_6 (g) + CO_2 (g) = $C_2H_5CO_2H$ (aq.)	$+11^a$
3.	C_3H_8 (g) + CO_2 (g) = $C_3H_7CO_2H$ (aq.)	$+11^a$
4.	CH_3CO_2H (aq.) + CO_2 (g) = $\begin{array}{l}CO_2H\\ \vert\\ CH_2\\ \vert\\ CO_2H \text{ (aq.)}\end{array}$	$+11^a$
5.	$C_2H_5CO_2H$ (aq.) + CO_2 (g) = $\begin{array}{l}CO_2H\\ \vert\\ (CH_2)_2\\ \vert\\ CO_2H \text{ (aq.)}\end{array}$	$+11^a$
6.	C_2H_5OH (aq.) + CO_2 (g) = $CH_3CHOHCO_2H$ (aq.)	11.2–12.6
7.	CH_3CHO (aq.) + CO_2 (g) = CH_3COCO_2H (aq.)	+14.8
8.	C_6H_6 (1) + CO_2 (g) = $C_6H_5CO_2H$ (aq.)	+ 7.1
9.	$C_{10}H_{12}$ (1) + CO_2 (g) = $C_{10}H_{11}CO_2H$ (S)	$+11^a$

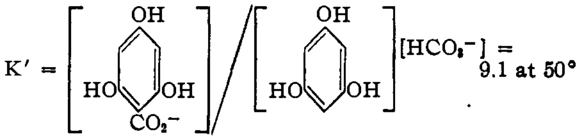
[a] Estimated by using the empirical equations of Parks and Huffman.[5]

It will be noticed in Table I that the free energy changes for the carboxylation of the aliphatic compounds are of the order of +10 kcal. or more. For benzene the ΔF is less unfavorable. Another aromatic carboxylation reaction for which there are quantitative data is

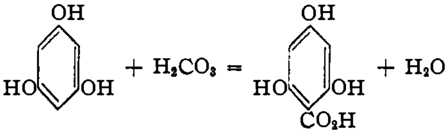


(2)

af Hällström[6] found the equilibrium constant



when the *partial pressure of CO_2 was 1 atmosphere.* Since phloroglucinolcarboxylic acid is a stronger acid ($K = 2.1$ at 25°)[7] than carbonic acid ($K_1 = 4.5 \cdot 10^{-7}$), the ΔF at 50° for



$$K'' = K' \cdot 4.5 \cdot 10^{-7}/2.1 = 1.95 \cdot 10^{-6} \qquad (3)$$

will be roughly +8.4 kcal.[8]

Because the carboxylation of aromatic compounds is less unfavorable than for aliphatics it

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(1) *Cf.*, Van Niel "Advances in Enzymology," Interscience Publishers, New York, N. Y., 1941, Vol. I, p. 263.

(2) Ruben, Kamen and Hassid, THIS JOURNAL, **62**, 3443 (1940).

(3) First suggested by Franck since the maximum rate of photosynthesis is attained (as shown by Emerson and Green, *Plant Physiol.*, **13**, 157 (1938)) at extraordinary low carbon dioxide partial pressures (~ 5.10^{-4} atmospheres).

(4) Unpublished work from this Laboratory.

(5) *Cf.*, W. M. Latimer, "The Oxidation States of the Elements and Their Potentials in Aqueous Solutions," Prentice-Hall, New York, N. Y., 1938. G. S. Parks and H. M. Huffman "Free Energies of Some Organic Compounds," Chemical Catalog Co., New York, N. Y., 1932.

(6) af Hällström, *Ber.*, **38**, 2288 (1905).

(7) Ostwald, *Z. physik. Chem.*, **3**, 241 (1889). In Beilstein, Vol. X, p. 468, K is listed as $2.1 \cdot 10^{-3}$ with Ostwald as reference. Yet in the original paper by Ostwald the value is given as 2.1.

(8) This is not exact since the dissociation constants at 50° are not at hand. It is likely this is not very much in error since from the the work of MacInnes and Belcher, THIS JOURNAL, **55**, 2630 (1930) and Hastings and Sendroy, *J. Biol. Chem.*, **65**, 445 (1925), the temperature coefficient seems small. The first constant for carbonic acid changes from $4.5 \cdot 10^{-7}$ at 25° to $4.68 \cdot 10^{-7}$ at 38°, and the second constant from $5.61 \cdot 10^{-11}$ to $6.03 \cdot 10^{-11}$.

has been suggested[2] that RH might contain polyphenols. (It is interesting to note that af Hällström investigated the phloroglucinol reaction at the suggestion of Luther for its possible connection with photosynthesis.) However, there are two rather serious objections to this proposal. In *Chlorella* the equilibrium constant[9] for (1) is $\sim 10^7$ greater than the constant for (3) and also a reasonable mechanism by which a carboxylated phenol is converted to carbohydrate is not readily formulated. From the above considerations it becomes apparent that the dark fixation of carbon dioxide in photosynthesis is complex and the positive free energy change for the carboxylation must be balanced by the occurrence of additional reactions involving other molecules or taking place within "R" itself.

A plausible explanation based upon recent advances in the study of phosphorylation[10] suggests itself and may be summarized in the following two equations

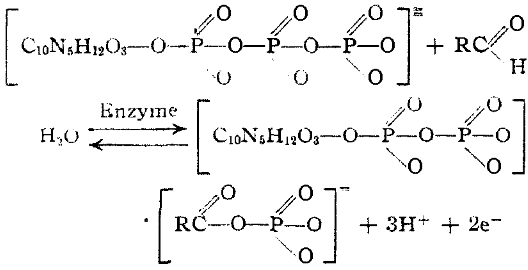
$$\text{Energy rich phosphate donor} + \text{RH} \xrightleftharpoons{\text{Enzyme}} \text{"free" donor} + \text{phosphorylated RH} \quad (4)$$

$$\text{Phosphorylated RH} + CO_2 \xrightleftharpoons{\text{Enzyme}} \text{RCOOH} + \text{inorganic phosphate} \quad (5)$$

Following Lipmann[10] the term energy rich phosphate donor is used to denote phosphorylated compounds for which ΔF is approximately -10 to -12 kcal. for the process

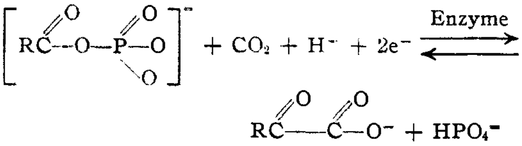
$$\text{Phosphate donor} \rightleftharpoons \text{free donor} + \text{inorganic phosphate} \quad (6)$$

If the carbon dioxide acceptor, RH, is an aldehyde (a suggestion for which we have some indirect evidence) and adenosine triphosphate is the energy rich phosphate donor, a specific illustration of equations (4) and (5) would be



$$\left[C_{10}N_5H_{12}O_3-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{|}}{P}}-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{|}}{P}}-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{|}}{P}}-O\right]^{\equiv} + RC\begin{matrix}\nearrow O\\ \searrow H\end{matrix} + H_2O \xrightleftharpoons{\text{Enzyme}} \left[C_{10}N_5H_{12}O_3-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{|}}{P}}-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{|}}{P}}-O\right]^{\equiv} + \left[R\overset{\overset{O}{\|}}{C}-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{|}}{P}}-O\right]^{=} + 3H^+ + 2e^- \quad (7)$$

$$\left[R\overset{\overset{O}{\|}}{C}-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{|}}{P}}-O\right]^{=} + CO_2 + H^- + 2e^- \xrightleftharpoons{\text{Enzyme}} R\overset{\overset{O}{\|}}{C}-\overset{\overset{O}{\|}}{C}-O^- + HPO_4^{=} \quad (8)$$

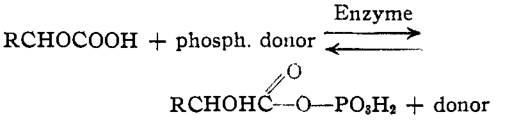


Reactions (7) and (8) involve not only phosphorylation and dephosphorylation but also oxidation and reduction. The electron acceptor in (7) and donor in (8) may well be a pyridine nucleotide.

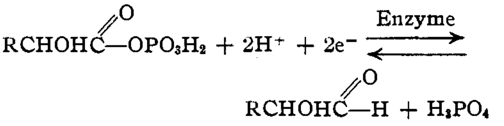
Thus by a sequence of coupled equilibria such as suggested above, the dark fixation of carbon dioxide may be accomplished (also suggested by Lipmann[10]) at the expense of the hydrolysis of an energy rich phosphorylated compound. It should be stated that the oxidation mechanisms of (a) 1-phosphoglyceraldehyde to 1-phosphoglyceric acid and (b) pyruvate to acetate and carbon dioxide which have been so brilliantly studied by Warburg[11] and Lipmann,[12] respectively, are the foundations upon which the above formulation is constructed.

Dark Reduction of Carbon Dioxide to Carbohydrate.—The reduction of RCOCOOH may be represented as follows

$$\text{RCOCOOH} + 2H^+ + 2e^- \xrightleftharpoons{\text{Enzyme}} \text{RCHOHCOOH} \quad (9)$$



$$\text{RCHOCOOH} + \text{phosph. donor} \xrightleftharpoons{\text{Enzyme}} \text{RCHOH}\overset{\overset{O}{\|}}{C}-O-PO_3H_2 + \text{donor} \quad (10)$$



$$\text{RCHOH}\overset{\overset{O}{\|}}{C}-OPO_3H_2 + 2H^+ + 2e^- \xrightleftharpoons{\text{Enzyme}} \text{RCHOH}\overset{\overset{O}{\|}}{C}-H + H_3PO_4 \quad (11)$$

RCHOHCHO (which may be considered to be a carbohydrate) may undergo (1) another cycle of phosphorylation, carboxylation and reduction, or (2) phosphorylation followed by cross linkage between identical or similar molecules to form polysaccharides in a manner similar to the polymerization of hexose 1-phosphate to starch and glycogen.[13,14] Reactions of the type in equations (9), (10) and (11) are well known.[10,15] The order 9, 10, 11 need not necessarily be unique. Equation (10) is introduced before (11) since the

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(9) We can deduce a lower limit for this equilibrium constant in the following manner. At a carbon dioxide partial pressure of 0.01 atm. RCO_2H comprises at least 1–2% by weight of the total organic material of a *Chlorella* cell. It follows then that $RH/RCO_2H < 100$ and $\therefore K > 1$.

(10) For two excellent reviews of this important subject see Lipmann, "Advances in Enzymology," *1941*, Vol. *1*, p. 137; and Kalckar, *Chem. Rev.*, **28**, 71 (1941).

(11) Warburg and Christian, *Biochem. Z.*, **303**, 40 (1939).

(12) Lipmann, Cold Spring Harbor Symposia on Quant. Biology, **VII**, 248 (1939).

(13) Cori, *Endocrinology*, **26**, 285 (1940); *J. Biol. Chem.*, **135**, 733 (1940).

(14) Hanes, *Proc. Roy. Soc.* (London), **B128**, 421 (1940); **B129**, 174 (1940).

(15) *Cf.*, "Mechanisms of Biological Oxidations," D. E. Green, Cambridge University Press, London, 1940.

reduction at pH 7 of carboxyl to aldehyde requires ~ 0.5 volt,[5] whereas the corresponding reduction of the phosphorylated anhydride to yield aldehyde and phosphoric acid requires ~ 0.3 volt.[10] The reduction of a carbonyl group to a secondary alcohol requires ~ 0.2 volt.[5] The reduction potential of diphosphorpyridine nucleotide is ~ 0.3 volt at pH 7.

Conversion of Radiant Energy to Chemical Energy.—Van Niel[1] has outlined[16] a plausible scheme for the conversion of radiant energy to chemical energy. For the sake of completeness it is desirable to point out that the proposals of Van Niel can be extended to provide for the rephosphorylation of the energy-rich phosphate donors used in equations (4) and (10) in addition to the production of reducing agents (*i. e.*, dihydropyridine nucleotides, etc.) and molecular oxygen. The photochemical process can be formulated as

$$\mathrm{A + B + H_2O} + h\nu \xrightleftharpoons{\text{chlorophyll}} \mathrm{AH + BOH\ (or\ AH_2 + BO)} \qquad (12)$$

AH (or AH_2) and BOH (or BO) merely symbolize reducing and oxidizing agents, respectively. AH-(AH_2) directly or indirectly effects the reduction in (9) and (10) whereas the energy stored in BOH (BO) can be used in part through a coupled[10,11,12] oxidoreduction reaction to form high energy phosphate donors (reverse of equation 4). Part of the BOH (BO) formed is oxidized to a peroxide (BO_2, etc.) and eventually yields molecular oxygen. The quantum yield is now known to be[17,18] 0.10–0.08 instead of 0.25, and it is evident that the 10–12 quanta are more than sufficient to fill the requirements of the above "theory."

Mechanisms of Carbon Dioxide Fixation and Reduction by Non-photosynthetic (*i. e.*, Chemosynthetic and Heterotrophic) Systems.—It has been suggested[19,20,21] that the reactions by which the carbon dioxide *is fixed and reduced* by photosynthetic and non-photosynthetic systems are essentially similar. Thus the suggestions outlined in this communication also afford a reasonable model for the mechanism by which these diverse organisms use the energy derived from the oxidation of various organic or inorganic substances to bring about the utilization of carbon dioxide.[22]

Support for this view is furnished by the recent important experiments of Vogler, Umbreit and co-workers[23,24] on the sulfur bacteria, *Thiobacillus thioxidans.*

Also it should be mentioned that Wood and Werkman,[25] as well as other workers, have presented evidence which indicates that in certain heterotrophic[25] organisms, a three-carbon compound (perhaps phosphorylated) is the carbon dioxide acceptor and the recent experiments of Krampitz, Wood and Werkman have furnished further encouraging support for the proposal.

The suggestions advanced here not only tend toward a more unified concept of carbon dioxide fixation and reduction, but more important, suggest experimentation along new lines.

I am indebted to Professor W. C. Bray and other members of this Laboratory for many helpful suggestions and criticisms.

Summary

In an earlier paper evidence was presented which indicated that the primary reaction in photosynthesis is a reversible thermal (dark) fixation of carbon dioxide represented by the equation $RH + CO_2 \rightleftarrows RCO_2H$.

A comparison of the equilibrium constant for the carbon dioxide fixation reaction in the green

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[16] As has Franck (*cf.* Franck and Herzfeld, *J. Phys. Chem.*, **45**, 978 (1941)).

[17] Manning, Stauffer, Duggar and Daniels, THIS JOURNAL, **60**, 266 (1938).

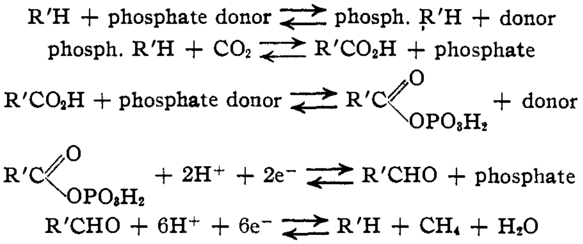
[18] Emerson and Lewis, *Am. J. Bot.*, **28**, 789 (1941).

[19] Thimann, *Science*, **88**, 506 (1938).

[20] Van Niel, Ruben, Carson, Kamen and Foster, *Proc. Natl. Acad. Sci.*, **28**, 8 (1942).

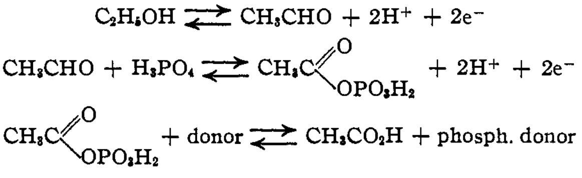
[21] Ruben and Kamen, THIS JOURNAL, **62**, 3451 (1941).

[22] For example, consider the methane bacteria (Barker, *Arch. Mikrobiol.*, **7**, 404 (1936)) which have been shown (Barker, Ruben and Kamen, *Proc. Natl. Acad. Sci.*, **26**, 426 (1940)) to reduce radioactive carbon dioxide to methane according to the equation $CO_2 + 2C_2H_5OH \rightarrow CH_4 + 2CH_3CO_2H$. Following the above model we can write the following steps for the formation of methane.



$$\mathrm{R'H + phosphate\ donor} \rightleftarrows \mathrm{phosph.\ R'H + donor}$$
$$\mathrm{phosph.\ R'H + CO_2} \rightleftarrows \mathrm{R'CO_2H + phosphate}$$
$$\mathrm{R'CO_2H + phosphate\ donor} \rightleftarrows \mathrm{R'C{\overset{\nearrow O}{\searrow OPO_3H_2}} + donor}$$
$$\mathrm{R'C{\overset{\nearrow O}{\searrow OPO_3H_2}}} + 2H^+ + 2e^- \rightleftarrows \mathrm{R'CHO + phosphate}$$
$$\mathrm{R'CHO} + 6H^+ + 6e^- \rightleftarrows \mathrm{R'H + CH_4 + H_2O}$$

and the energy yielding reactions as



$$\mathrm{C_2H_5OH} \rightleftarrows \mathrm{CH_3CHO} + 2H^+ + 2e^-$$
$$\mathrm{CH_3CHO + H_3PO_4} \rightleftarrows \mathrm{CH_3C{\overset{\nearrow O}{\searrow OPO_3H_2}}} + 2H^+ + 2e^-$$
$$\mathrm{CH_3C{\overset{\nearrow O}{\searrow OPO_3H_2}} + donor} \rightleftarrows \mathrm{CH_3CO_2H + phosph.\ donor}$$

[23] Vogler, Le Page and Umbreit, *J. Gen. Physiol.*, **26**, 89 (1942).

[24] Vogler, *ibid.*, **26**, 103 (1942).

[25] For a review of carbon dioxide fixation by heterotrophic systems see Wood and Werkman, *Bot. Rev.*, **8**, 1, 1942, and "Advances in Enzymology." **II**, 139 (1942).

alga *Chlorella pyrenoidosa* with the known equilibrium constants for simple carboxylation reactions of this general type shows a difference in order of magnitude of something like 10^7. It appears therefore that the primary reaction of carbon dioxide in photosynthesis does not consist solely of carboxylation but must be a more complex sequence of reactions.

A new formulation of the mechanism of photosynthesis is briefly presented which offers a plausible model for the fixation and reduction of carbon dioxide not only for green plant photosynthesis but also for carbon dioxide fixation and reduction by the many different chemosynthetic and heterotropic organisms.

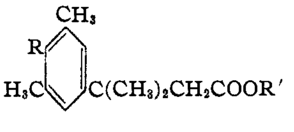
BERKELEY, CALIF. RECEIVED JUNE 16, 1942

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

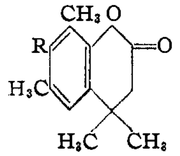
The Nitration of β-Polyalkylphenylisovaleric Acids. II. β-[3,5-Dimethylphenyl]-isovaleric Acid[1]

BY LEE IRVIN SMITH AND LEO J. SPILLANE[2]

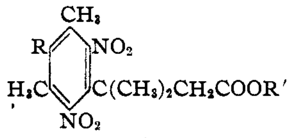
Nitration of methyl β-[3,4,5-trimethylphenyl]-isovalerate I at low temperatures, by action of potassium nitrate and sulfuric acid in the presence of chloroform, has been shown to give two crystalline compounds in almost equal amounts. The structure of one of these was established as the nitrohydrocoumarin II; but the structure of the other was not established. This second substance gave analytical values well in accord with those required by III, the dinitro derivative of I, but the chemical behavior of the substance was so peculiar that structure III appeared rather unlikely.[1] In order to investigate further this peculiar reaction, and to gain more information concerning the structure of the compounds formed, a study has been made of the nitration of a lower homolog of I, methyl β-[3,5-dimethylphenyl]-isovalerate IV and the corresponding acid V.[3]



I, R = R' = CH₃
IV, R = H, R' = CH₃
V, R = R' = H

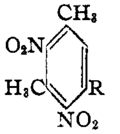


II, R = CH₃
VIII, R = NO₂
X, R = NH₂



III, R = R' = CH₃
VI, R = NO₂, R' = CH₃
VII, R = NO₂, R' = H

Nitration of a chloroform solution of the ester IV by action of potassium nitrate and sulfuric acid gave a mixture of two products. The major part (79%) consisted of an oily nitrosulfonic acid, and the remainder was a crystalline compound which had the composition $C_{14}H_{18}O_6N_2$, a dinitro derivative of IV.



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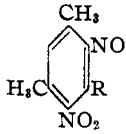
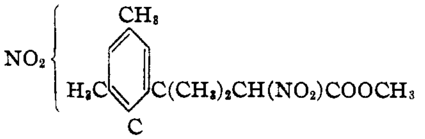


B

R = —C(CH₃)₂CH₂COOCH₃ (H)



C

When the ester IV was nitrated at room temperature by action of sulfuric acid and fuming nitric acid, an excellent yield of a crystalline trinitro ester (VI) was obtained, together with a small amount of a trinitro acid (VII). Further nitration of the dinitro ester produced VI; moreover, the trinitro ester VI could be hydrolyzed to the trinitro acid VII, and the latter could be reconverted into the ester VI by action of methanol and sulfuric acid. All of these nitro compounds, when warmed with alkali, gave cherry-red solutions.[4] It appeared, therefore, that the structure of these dinitro compounds was most likely A or B, but in view of the very peculiar properties of the trinitro ester III, structure C also had to be considered.

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[1] Paper I, THIS JOURNAL, **62**, 780 (1940).

[2] Abstracted from a thesis by L. J. Spillane, presented to the Graduate Faculty of the University of Minnesota, in partial fulfillment of the requirements for the Ph.D. degree, July, 1942.

[3] Smith and Spillane, THIS JOURNAL, **65**, 202 (1943).

[4] Hoffman, *ibid.*, **51**, 2542 (1929), noted a similar behavior when the nitro derivative of methyl β-phenyl isobutyl ketone was dissolved in alkali.