cyanide, phenylurethan and ultraviolet light) administered in controlled dosage so that photosynthesis is either partially or completely stopped while respiration is unaffected have been studied. The light and dark C^*O_2 reductions show the same sensitivity as normal photosynthesis to these widely different modes of injury.

5. The dark assimilation is reversible and apparently independent of the chlorophyll concentration.

6. *Chlorella pyrenoidosa*, which has a higher rate of photosynthesis than *Chlorella vulgaris*, also possesses a greater capacity for assimilating C^*O_2 in the dark. This is in agreement with the suggestion that the dark C^*O_2 reduction may be the primary step in photosynthesis.

7. Chemical tests on the water-soluble material formed in the light and the dark indicate the presence of at least one alcoholic hydroxyl and one carboxyl group in the active molecules. Attempts to identify the active compound as one of a large number of organic substances known to exist in plants (*i. e.*, sugars, aldehydes, ketones, proteins, etc.) were not successful.

8. The major part, if not all, of the C^*O_2 taken up in the dark was found in carboxyl groups. A smaller but appreciable fraction of the C^*O_2 reduced in the light was in COOH groups.

9. No radioactive carbon was found in formaldehyde (and other volatile substances) even after very short (one minute) exposures to C^*O_2.

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[Contribution from the Department of Chemistry, University of California, the Radiation Laboratory, University of California, and the Department of Chemistry, Stanford University]

Photosynthesis with Radioactive Carbon. III. Ultracentrifugation of Intermediate Products

By S. Ruben, M. D. Kamen and L. H. Perry

Considerable progress toward a solution of the problem of photosynthesis will be achieved when the identity of the molecules formed in the intermediate stages of carbon dioxide reduction is known. It is the object of this work to isolate and identify the primary products formed by the dark and light reduction of carbon dioxide using the radioactive isotopes of carbon as tracers. Since many specific chemical tests[1] have been unsuccessful, a more general method of attack is necessary. Knowledge of the molecular weight is the first objective.

A survey of the various methods available for molecular weight determinations indicated the most satisfactory are based upon sedimentation and diffusion properties. With the ultracentrifuge molecular weights can be found by (1) the sedimentation velocity or (2) sedimentation equilibrium. For sedimentation equilibrium spinning for long periods (days) is required and therefore is excluded,[2] since the half-life of C^{11} is only twenty-one minutes. The sedimentation velocity method which involves rotation periods of the order of an hour was used. The transparent ultracentrifuge is most widely used, the concentration gradient being measured by optical methods while the top is in rotation. This technique cannot be employed in the C* work since the radioactive molecular species are present only at high dilution. The sedimentation must be followed by radioactivity measurements. The opaque analytical ultracentrifuge[3,4] developed by McBain and his students is particularly suited for this problem because the top can be stopped and a sample from the periphery removed for analysis.

After ordinary centrifugation the supernatant aqueous extract from *Chlorella* exposed to C^*O_2 was filtered through a no. 4 Jena sintered glass disk. This solution was introduced into a 37-mm. rotor according to the method described by McBain and Leyda.[4] The possibility of spurious sedimentation due to adsorption on the metallic immobilizers was eliminated in experiments in which convection currents were set up in the top. No change ($< 0.5\%$) in the specific activity could be detected, indicating no measurable adsorption. The results of five successful runs are summarized in Table I.[5]

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[1] *Cf.* preceding paper, This Journal, **62**, 3443 (1940).

[2] The production of strong samples of long-lived radio-carbon will make possible these and other long term experiments: *cf.* Ruben and Kamen, *Phys. Rev.*, **57**, 549 (1940).

[3] McBain, *Chem. Rev.*, **24**, 289 (1939).

[4] McBain and Leyda, This Journal, **60**, 2998 (1938).

[5] Several of these runs were carried out at the Shell Development Company at Emeryville. We are grateful to Dr. T. F. Ford and Mr. D. C. Waldman for their help and coöperation.

TABLE I

Conditions under which algae reduced C^*O_2	Velocity of rotor, r. p. s.	Time of rotation, min.	Increase in specific activity at periphery, %	Sedimentation velocity constant ($\times 10^{-14}$)
4 Min. in light	2250	85	15	6.2[a]
10 Min. in light	2250	90	15.8	6.1
10 Min. in light	2400	60	11.1	5.7
20 Min. in light	2000	120	12	7.5
20 Min. in dark	2250	70	16	8.6

[a] Sedimentation carried out in KBr solution ($\rho = 1.22$ g./cc.).

The average of the four values (for $\rho = 1$) is 7.0×10^{-14} c. g. s. unit. Unfortunately any trend in the velocity constants with photosynthetic conditions cannot be considered significant because of experimental error. It is of interest to compare the above values with the velocity constant of sucrose. A 1% solution of sucrose was spun for ninety minutes at 2250 r. p. s. The increase in concentration[6] at the periphery was 4% yielding a velocity constant of 1.7×10^{-14}. This is the value obtained by Leyda.[3] The theoretical value according to McBain[3] for anhydrous sucrose is 2.3×10^{-14}.

We are indebted to Professor W. C. Bray and Professor J. W. McBain for their interest and advice. Thanks are due to Mr. G. F. Nelson and Mr. G. T. Boies for mechanical assistance and many suggestions. The coöperation of Professor E. O. Lawrence and members of the Radiation Laboratory made these experiments possible. Financial aid from the Rockefeller Foundation to the Radiation Laboratory is gratefully acknowledged.

Summary

Using radioactive carbon as an indicator the sedimentation velocity constant of the radioactive molecule(s) formed by *Chlorella pyrenoidosa* in the light and dark has been found to have an average value of 7.0×10^{-14}. The velocity constant for sucrose obtained under the same conditions is 1.7×10^{-14}. This indicates the molecular weight is about four times that of sucrose, assuming other factors equal.

The additional data necessary for a more accurate molecular weight evaluation are presented in the following paper.

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[6] We are indebted to Dr. W. Z. Hassid for the sucrose analyses and the chlorella cultures.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND THE RADIATION LABORATORY, UNIVERSITY OF CALIFORNIA]

Photosynthesis with Radioactive Carbon. IV. Molecular Weight of the Intermediate Products and a Tentative Theory of Photosynthesis

BY S. RUBEN AND M. D. KAMEN

It is the purpose of this paper to describe the diffusion experiments which in conjunction with the determination of the sedimentation velocity constant[1] make possible a calculation of the approximate[2] molecular weight of the primary radioactive products.

The molecular weight of a compound can be calculated from a knowledge of the diffusion coefficient and the sedimentation velocity constant. The relation is

$$M = \frac{RTs}{D(1 - \rho\overline{V})} \qquad (1)$$

where M is molecular weight, R gas constant, T temperature, s sedimentation velocity constant, D diffusion coefficient, $\overline{V}$ partial specific volume of solute, ρ density of solution. The velocity constant was determined with the McBain opaque ultracentrifuge.[1] The diffusion coefficient was measured by the sintered glass plate technique.[3] The sensitivity of the method was increased by the use of small volumes of solution behind the sintered diaphragm (no. 4 Jena). The cells were calibrated with 1 N potassium chloride and showed no streaming effects when tilted. The diffusion coefficients of sucrose and sodium chloride were measured and agreed well with the values in the literature.[4,5]

Chlorella cells, after exposure to C^*O_2 in the light or dark for various periods, were boiled gently for approximately one minute, centrifuged and the supernatant extract filtered through a no. 4 Jena glass filter. This filtrate was introduced by gentle suction into the diffusion cell. De-

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[1] Cf. III of this series, THIS JOURNAL, **62**, 3450 (1940).

[2] Due to experimental difficulties (short half-life of C^{11}, etc.) it is not practical to obtain high accuracy but rather to set a lower limit on the molecular weight.

[3] Northrup and Anson, *J. Gen. Physiol.*, **12**, 543 (1929).

[4] Friedman and Carpenter, THIS JOURNAL, **61**, 1745 (1939).

[5] Mehl and Schmidt, *Univ. Calif. Pub. Physiol.*, **8**, 165 (1937).