

Summary

The flow of liquid helium through small channels has been studied to obtain data desired in connection with the design of apparatus for adiabatic demagnetization experiments.

Formulas have been developed for the case of isothermal laminar flow when the material enters as a liquid and leaves as a gas.

The viscosity of liquid helium was found to de-

crease from 2.0×10^{-5} poise at 2.266°K . to 1.0×10^{-7} poise at 1.468°K . The largest Reynolds number, which occurred at 1.468°K ., was estimated to be about 200. At the lowest temperature of measurement the viscosity is still decreasing rapidly and appears to be approaching a value of zero at the absolute zero of temperature.

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

Radioactive Carbon in the Study of Photosynthesis

BY S. RUBEN, W. Z. HASSID AND M. D. KAMEN

In this preliminary report, there are presented some results obtained in the study of photosynthesis by the use of radioactive carbon, C^{11} , prepared in the Berkeley cyclotron. Experiments on barley plants were conducted under conditions of light and darkness as indicated below. Radioactive carbohydrates and chlorophyll (as phytochlorin and phytorhodin) formed in the living leaf on exposure to C^*O_2 were isolated.

Despite the 21.5 ± 0.5 minutes² half-life of C^{11} , the yields obtainable by bombarding amorphous boron³ with 8 MEV. deuterons are such as to make this method feasible. The nuclear reactions are ${}_1\text{D}^2 + {}_5\text{B}^{10} \rightarrow {}_6\text{C}^{11} + {}_0n^1$ and ${}_6\text{C}^{11} \rightarrow {}_5\text{B}^{11} + e^+$. The initial intensity was 10^7 – 10^8 times the background of the Geiger counter used. The boron target was bombarded in a gas-tight chamber incorporating many features found to be of value in the Radiation Laboratory for the handling of high intensity beams.

The gas in the target holder was swept through a heated combustion tube containing cupric oxide into large Pyrex desiccators containing the plants. The bombarded boron was then introduced into the combustion tube and intensely heated in a stream of air. To facilitate the removal of the trapped radio-carbon a small piece of filter paper was placed in contact with the boron. The light source was a 500-watt floodlight directly above the desiccators and the temperature was kept at 28 – 30° .

The isolation of the water soluble radioactive carbohydrates was performed as follows. Immediately after removal from the C^*O_2 atmosphere, the plant was cut into small bits and immersed in boiling 80% ethanol for five

minutes.⁴ After filtration the extract was boiled to remove the alcohol. The radioactivity of the solution as measured by means of a small Geiger counter varied between 10^5 and 10^6 counts/min. at the time of measurement depending on the conditions of the experiment, the radioactivity of the solution being measured by pipetting out 1 or 2 cc. on a small piece of blotter (5×8 cm.) which then was partially dried over a hot-plate, covered with cellophane and wrapped around a small aluminum Geiger counter with 0.1 mm. wall thickness. This was found to be the most convenient and sensitive method. The following experiment was used to show that the activity was not caused by dissolved C^*O_2 . Sodium bicarbonate solution was added, followed by dilute acetic acid and the solution was then boiled vigorously. No detectable change in the radioactive content of the solution was produced.

Pigments as well as insoluble lead salts were then removed by the addition of charcoal and lead acetate, and filtration through talc. Thirty to forty per cent. of the C^* content of the solution was carried down by this precipitate.

Aliquots of the colorless filtrate were taken and osazones precipitated. Both the radioactivity of the simple reducing sugars (*i. e.*, those precipitable directly by phenylhydrazine) and that of the more complex carbohydrates (the osazone precipitation preceded by hydrolysis with boiling 1 *N* hydrochloric acid for three minutes) were measured. The crystalline osazone precipitates, after being carefully washed with water, dried and covered with cellophane, were wrapped around the Geiger counter. The radioactivity of the alcohol-water insoluble material, mainly cellulose, was determined by dissolving the plant residue in sulfuric acid.

The methods (Tswett absorption column, etc.) in use at the present time for a rapid isolation of chemically unaltered chlorophyll are not such as to preclude minute traces of colorless impurities. It was deemed necessary, therefore, to isolate a chlorophyll derivative.

(1) The symbol C^* will be used to represent carbon labeled by the radioactivity of some of its atoms.

(2) Determined in this Laboratory.

(3) We are indebted to Professor E. D. Eastman for the boron used in these experiments.

(4) From 90 to 95% of the water-soluble carbohydrates are removed by this procedure.

TABLE I

Expt.	Previous treatment	Exposed to C*O ₂	C*O ₂ content of gas phase	Radioactivity of alcohol-water-sol. fraction	Water-soluble carbohydrate		Percentage of C fixed as Phytochlorin and phytorhodin		Water insoluble material
					Simple	Total	Total		
1	Light ~1 hour	15 min. light	~1%	5 × 10 ⁶	5	18			
2	Light ~1 hour	30 min. light	~1%	9 × 10 ⁶	3	21	0.04		
3	Light ~1 hour	50 min. light	~1%	13 × 10 ⁶	4	23			
4	Light ~1 hour	70 min. light	~1%	19 × 10 ⁶	4	25			
5	Dark ~20 hrs.	40 min. light	~1%	2 × 10 ⁶	3	11	.08		
6	Light ~1 hour	60 min. light	~3%	100 × 10 ⁶		5			10
7	Light ~1 hour	15 min. dark	~3%	2.5 × 10 ⁶		0.7			
8	Light ~1 hour	30 min. dark	~3%	3.5 × 10 ⁶		0.4	0(<0.003)		
9	Dark ~3 hrs.	15 min. dark	~3%	3 × 10 ⁶		0(<0.05)			0(<0.1)

The phytochlorin and phytorhodin isolation was accomplished as follows: after treatment with C*O₂, the leaves were finely ground with sand and extracted with anhydrous acetone and then ether until the material was practically colorless. This extract, after filtration, was poured into a large separatory funnel and washed twice with water, followed by dilute sodium hydroxide to remove flavins and plant acids. The ether layer was evaporated to dryness, a concentrated solution of sodium hydroxide in methyl alcohol was added, and the solution was boiled gently for a minute to saponify the chlorophyll. After the addition of water, the solution was extracted with ether to remove the yellow pigments and any unsaponified esters. The water layer was neutralized and then extracted with ether. The ethereal solution was then extracted with 12% hydrochloric acid and the aqueous extract was neutralized with ammonium hydroxide and extracted with ether. This serves to remove alcohols and fatty acids. The ether layer containing the phytochlorin and phytorhodin was washed several times with water and its C* content measured. The aqueous washings were always inactive.

The experiments and results are summarized in the accompanying table. Owing to the complexity of the plant system, accurate data are difficult to obtain without carrying out an unduly large number of experiments. The values in the accompanying table represent for the most part the average of only 2 or 3 separate experiments and consequently must be considered approximate.

Since the labeled carbon found in the chlorophyll was but 0.04–0.08% of the C* fixed, every precaution was exercised to eliminate contamination from the phytochlorin and phytorhodin fraction. The fact that in experiment 8 no radioactivity was found⁵ in the fraction isolated from leaves exposed to C*O₂ in the dark furnishes a convincing check of the chemical procedure employed. While the amount of labeled carbon incorporated in the saponified chlorophyll is only ~1/600 of that found in the water soluble carbohydrates, this ratio becomes ~1/4 when allowance is made for the high molal ratio of carbohydrate to chlorophyll.

(5) 0.003% could have been detected.

Experiments 7 and 8 indicate C*O₂ "reduction" and the formation of some labeled carbohydrate in the absence of light. When in experiment 9 leaves were kept in the dark for two and one-half to three hours prior to the admission of C*O₂ no (<0.05%) radioactive carbohydrates could be detected. The possibility of C*O₂ "reduction" by ordinary chemical interchange has been tested experimentally and can be excluded. In separate experiments C*O₂ was shaken with glucose, sucrose, dried leaves, and several plant extracts obtained from barley leaves by treatment with cold as well as boiling 80% alcohol. No interchange could be detected in any of these cases.

The "dark reduction" of C*O₂ may be due to interchange with the CO₂ produced in the respiratory (oxidation) process. If this explanation is correct it is necessary to assume that at least some of the reactions involved in the oxidation process are reversible. The greater part of the non-carbohydrate radioactive material formed in the light could also be accounted for by such an interchange.

C*O₂ has been found to interchange with cell-free sap, obtained from fresh barley leaves. The amount of C*O₂ "reduced" by the sap is ~5% of the "dark reduction" by an equivalent quantity of intact leaves.

In addition, a non-photosynthetic respiring system (yeast) has been found to interchange with C*O₂. Furthermore, the dark interchange of C*O₂ with barley as well as yeast is markedly reduced by the presence of hydrogen cyanide. It would seem that interchange is dependent upon the normal metabolic processes or at least the presence of certain active enzymes.

The bulk of the labeled carbon found in the various plant extracts is not carbohydrate, or carbonate. This fraction is being investigated. The keto acids such as pyruvic, etc., do not contain any labeled carbon (*i. e.*, less than 0.01%). They were precipitated as insoluble hydrazones in

0.5 *N* hydrochloric acid by 2,4-dinitrophenylhydrazine. Pyruvic acid was added as a carrier.

Experiments with chlorella are in progress in which the $C^{14}O_2$ uptake will be measured simultaneously by gas as well as radioactive analysis.

The heavy carbon isotope, C^{13} , when made available in sufficient quantity, undoubtedly will be capable of wider application than the short-lived radio-carbon used in these experiments.

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Summary

Using short lived radioactive carbon, C^{14} , as an indicator, the assimilation of $C^{14}O_2$ by barley has been studied. $C^{14}O_2$ fixation has been observed both in the absence and presence of light.

Leaves kept in complete darkness as well as illuminated leaves formed radioactive carbohydrates. When, however, leaves were placed in the dark for two and one-half to three hours prior to the administration of $C^{14}O_2$ the formation in the absence of light of radioactive carbohydrates could not be detected. The chlorophyll contained radioactivity after exposure to $C^{14}O_2$ in the light but not after exposure in the dark.

The bulk of the radioactive material found in the plant is water soluble and is not carbohydrate, carbonate, keto acids, or pigments.⁶

These results seem to indicate that the cell contains substances, either directly involved in photosynthesis or in respiration, which react with $C^{14}O_2$ reversibly in a non-photochemical process.

A more complete report of these and other experiments now in progress will be reported later.

(6) Note added in proof: experiments in progress demonstrate that in the green algae, chlorella, a considerable fraction of the radioactive substances formed is also non-carbohydrate.

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The Condensation of Propene and Isobutene with Benzene in the Presence of Anhydrous Ferric Chloride

BY W. M. POTTS AND L. L. CARPENTER¹

The reaction of alkenes with aromatic hydrocarbons in the presence of anhydrous metallic halides to form alkylated aromatic hydrocarbons was first observed by Balsohn.² Alkylation with ethene and propene in the presence of aluminum chloride³ and propene and butene with boron fluoride⁴ has been investigated. Ferric chloride usually has been found less satisfactory in the Friedel and Crafts reaction than aluminum chloride, but it has been found more satisfactory in the preparation of ketones.⁵ Fractions of cracked gasoline⁶ were condensed with benzene using ferric chloride and monoalkylated benzenes were obtained.

(1) From a thesis by L. L. Carpenter submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering.

(2) Balsohn, *Bull. soc. chim.*, [2] **31**, 539 (1879).

(3) Berry and Reid, *THIS JOURNAL*, **49**, 3142 (1927).

(4) Slanina, Sowa and Nieuwland, *ibid.*, **57**, 1547 (1935).

(5) Nencki, *Ber.*, **30**, 1766 (1897); Meissel, *ibid.*, **32**, 2419 (1899).

(6) Kuryndin, Voevodova and Rosskazova, *J. Applied Chem. (U. S. S. R.)*, **10**, 877 (1937).

In this investigation, propene and isobutene were condensed with benzene using anhydrous ferric chloride as the catalyst. The ferric chloride was sublimed directly into the reaction flask. Propene was obtained by dehydrating isopropyl alcohol with phosphorus pentoxide and isobutene was prepared by heating *t*-butyl alcohol with oxalic acid. There was no evidence of a reaction between the anhydrous ferric chloride and benzene, but heat was evolved upon the passage of the alkene into the well-stirred mixture. The resulting mixture was filtered and the filtrate distilled using a Snyder⁷ eight-bulb column.

Milligan and Reid⁸ reported an optimum temperature of 70–90° for the condensation of ethene with benzene using aluminum chloride, but with propene and benzene at 80° using ferric chloride, there was no condensation. This indicates a lower optimum temperature for ferric chloride. With an excess of benzene, very little diisopropylbenzene is formed. Equimolar quantities of isobutene and benzene with ferric chloride yield *t*-butylbenzene principally; two moles of isobutene to one of benzene yields 1,4-di-*t*-butyl-

(7) Hill and Ferris, *Ind. Eng. Chem.*, **19**, 379 (1927).

(8) Milligan and Reid, *THIS JOURNAL*, **44**, 206 (1922).