Heavy Oxygen (O^{18}) as a Tracer in the Study of Photosynthesis

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It is generally agreed that the net reaction for green plant photosynthesis can be represented by the equation

$$CO_2 + H_2O + h\nu \xrightarrow{\text{Chlorophyll}} O_2 + (1/n)(C \cdot H_2O)n \quad (1)$$

and also that very little is known about the actual mechanism. It would be of considerable interest to know how and from what substance the oxygen is produced. Using O^{18} as a tracer we have found that the oxygen evolved in photosynthesis comes from water rather than from the carbon dioxide.

The heavy oxygen water used in these experiments was prepared by fractional distillation[1] and was distilled from alkaline permanganate before use. The isotopic oxygen content was determined by the method of Cohn and Urey[2] using carbon dioxide and a mass spectrometer. Heavy oxygen carbonate was prepared by allowing a solution of potassium acid carbonate ($KHCO_3$) in heavy oxygen water to come to approximate isotopic equilibrium, adding a nearly equivalent quantity of potassium hydroxide and distilling off the water, finally drying in an oven at 120°. Isotopic analysis of this carbonate or of the carbonate in a solution, was performed by rendering the solution sufficiently alkaline to prevent exchange[3] and precipitating calcium carbonate. The calcium carbonate after filtering, washing and drying at 120°, was calcined at red heat in an evacuated platinum bulb connected to the gas handling system of the mass spectrometer, and the evolved carbon dioxide analyzed for heavy oxygen.

Young active *Chlorella* cells were suspended in heavy oxygen water (0.85% O^{18}) containing ordinary potassium bicarbonate and carbonate. Under these conditions the oxygen exchange between the water and bicarbonate ion is slow and readily measurable.[3] The isotopic ratio in the evolved oxygen was measured with a mass spectrometer. In other experiments the algae were allowed to carry on photosynthesis in ordinary water and heavy oxygen potassium bicarbonate and carbonate. The results of these experiments are summarized in Table I.

TABLE I

ISOTOPIC RATIO IN OXYGEN EVOLVED IN PHOTOSYNTHESIS BY *Chlorella*[a]

Expt.	Substrate	Time between dissolving $KHCO_3$ + K_2CO_3 and start of O_2 collection, minutes	Time at end of O_2 collection, minutes	Percent. O^{18} in H_2O	Percent. O^{18} in HCO_3^- + $CO_3^=$	Percent. O^{18} in O_2
1	0.09 *M*	0		0.85	0.20	..
	$KHCO_3$	45	110	.85	.41[b]	0.84
	+0.09 *M*	110	225	.85	.55[b]	.85
	K_2CO_3	225	350	.85	.61	.86
2	0.14 *M*	0		.20		..
	$KHCO_3$	40	110	.20	.50	.20
	+0.06 *M*	110	185	.20	.40	.20
	K_2CO_3					
3	0.06 *M*	0		.20	.68	..
	$KHCO_3$	10	50	.20		.21
	+0.14 *M*	50	165	.20	.57	.20
	K_2CO_3					

[a] The volume of evolved oxygen was large compared to the amount of atmospheric oxygen present at the beginning of the experiment. [b] These are calculated values.

It is apparent that the O^{18}/O^{16} ratio of the evolved oxygen is identical with that of the water. Since the oxygen in OH, COOH, O—O, C=O, etc., groups exchanges but very slowly[4] with water at room temperature and moderate *p*H, it seems reasonable to conclude that the oxygen originates solely from the water. While this conclusion makes it possible to reject many of the suggestions proposed in the past[5] it does not enable a choice to be made between the several more recent hypotheses. However it is of interest to note that van Niel[6] has specifically suggested that the oxygen may arise by a dehydrogenation of water.

We have also attempted to ascertain whether the evolution of oxygen was a reversible reaction. The algae were suspended in ordinary potassium bicarbonate and carbonate solution and photosynthesis allowed to proceed in the presence of heavy oxygen. In other experiments the algae evolved heavy oxygen in the presence of light oxygen. The results are shown in Table II.

TABLE II

ISOTROPIC RATIO IN OXYGEN EVOLVED IN PHOTOSYNTHESIS BY *Chlorella* IN PRESENCE OF OXYGEN

O_2 present in gas space at beginning, ml.	O_2 produced in photosynthesis by 200 mm.³ algae, ml.	Per cent. O^{18} at *end of experiment* Obsd.	Per cent. O^{18} at *end of experiment* Calcd. for no exchange
2.29 (O^{18} = 0.20%)	1.55 (O^{18} = 0.85%)	0.43	0.46
3.64 (O^{18} = .20%)	1.18 (O^{18} = .85%)	.34	.36
1.44 (O^{18} = .85%)	0.73 (O^{18} = .20%)	.59	.62
4.81 (O^{18} = .85%)	1.22 (O^{18} = .20%)	.69	.71

There is no indication of exchange reactions involving oxygen. The experimental errors are such that an exchange involving less than $5 \cdot 10^{-8}$ mol of oxygen with each cu. mm. of algae would not be detected.

Similar experiments with *Chlorella* and yeast were performed in order to determine whether the oxidation (respiration) reactions utilizing oxygen

(1) Randall and Webb, *Ind. Eng. Chem.*, **31**, 227 (1939).

(2) Cohn and Urey, THIS JOURNAL, **60**, 679 (1938).

(3) Mills and Urey, *ibid.*, **62**, 1019 (1940).

(4) For a review of oxygen exchange reactions see Reitz, *Z. Elektrochem.*, **45**, 100 (1939).

(5) For an excellent review of this subject up to 1926 see H. A. Spoehr "Photosynthesis," Chem. Cat. Co., New York, N. Y., 1926.

(6) Van Niel, *Cold Spring Harbor Symposia on Quant. Biol.*, **3**, 138 (1935).

were reversible. The results are summarized in Table III.

TABLE III

ISOTOPIC OXYGEN RATIO IN RESPIRATION WITH *Chlorella* AND YEAST

Respiring system	O_2 (O^{18} = 0.85%) present at start of expt., ml.	O_2 utilized in respiration, ml.	Per cent. O^{18} in O_2 at end of experiment Obsd.	For no exchange
Chlorella cells in dark for 90 minutes	0.82	0.11	0.85	0.85
Yeast cells for 60 minutes	4.5	2.4	.84	.85

Here also there is no indication for an exchange reaction involving molecular oxygen.

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