INFLUENCE OF DEUTERIUM OXIDE ON THE RATE OF PHOTOSYNTHESIS

A STUDY of the influence of heavy water on photosynthesis is of particular interest, not only on account of the fundamental importance of the process, but also because water enters directly into the chemical reaction. Recently we have measured the rate of photosynthesis when D_2O is used instead of H_2O.

The rate of O_2 evolution by Chlorella suspended in a carbonate-bicarbonate buffer was measured by means of Warburg manometers.[1] The illumination intensity was 2,000 meter candles, and the temperature was 25.7° C. The Chlorella was cultured in flasks by the usual method. For an experiment equal volumes of the culture were withdrawn and centrifuged, and the supernatant liquid was decanted. The cells were then washed several times by adding water, centrifuging and decanting. To one sample were added 5 cc of H_2O. To the other were added 5 cc of 100 per cent. D_2O (the resulting mixture being about 97 per cent. D_2O). Both were shaken and allowed to stand for 30 minutes. The water was then removed, after centrifuging, by decantation, and the buffer (as a rule Warburg's No. 9) was added to the moist cells. One buffer was made with H_2O and the other with 100 per cent. D_2O; due to water clinging to the cells, the latter buffer was diluted to about 99.9 per cent. Measurements were made within the first hour after the buffer had been added. Different cultures were used in the various experiments.

Table 1 gives the results. All the rates have been corrected for respiration except those in experiments 1 and 2. The correction, obtained experimentally, amounted to from 5 to 10 per cent. of the rate of photosynthesis.

TABLE 1

Exp.	Buffer	O_2 evolved in cmm/min./vessel	Ratio
1*	No. 9 H_2O	2.77	1.05
	No. 9 H_2O	2.63	
2	No. 9 D_2O	1.17	0.38
	No. 9 H_2O	3.10	
3	No. 9 D_2O	1.27	0.45
	No. 9 H_2O	2.84	
4	No. 9 H_2O	3.08	1.01
	No. 9 H_2O	3.05	
5	No. 7.5 D_2O	1.20	0.39
	No. 7.5 H_2O	3.04	
	No. 9 D_2O	1.30	0.42
	No. 9 H_2O	3.12	

* Exp. 1 indicates the precision of duplicate tests. There were approximately 120 million cells per vessel.

[1] O. Warburg, *Biochem. Zeit.*, 100: 230–270, 1919.

In experiment 3 the cells were allowed to remain for 16 hours in 97 per cent. D_2O before being transferred to the buffer; it may be seen that the rate of photosynthesis, relative to that for cells treated similarly with H_2O, is about the same as when the period of preliminary soaking was only 30 minutes. In experiment 4 the cells were soaked for 15 hours in 97 per cent. D_2O and, after removal of this, for 4 hours in ordinary water. The relative rates show that contact with D_2O for this period of time does not permanently injure the cells.

A comparison is made in experiment 5 of the rates obtained with buffer No. 9 and buffer No. 7.5 (the latter providing one third the CO_2 concentration of the former). The results show that a marked lowering of the CO_2 concentration causes only a slight decrease in the rate of photosynthesis. This indicates that the curves for rate of photosynthesis as a function of CO_2 concentration, for both H_2O and D_2O, are relatively flat in the range of CO_2 concentration employed. Therefore, the difference in the rates observed when using D_2O and H_2O buffers of the same molar concentration can hardly be due to differences in the CO_2 concentration in the two buffers.

Thus our experiments show that, when measured directly, the rate of photosynthesis with 99.9 per cent. D_2O is about 0.41 of that with ordinary H_2O. From chemical analyses of algae cultured in heavy water, Reitz and Bonhoeffer[2] estimated that the velocity constant for assimilation of D was about 0.43 that for assimilation of H.

The difference in rates of photosynthesis with D_2O and H_2O can not as yet be explained in terms of the mechanism of the reaction, but we are continuing our experiments with this object in view. The use of D_2O provides a new experimental approach to the problem of establishing a generally satisfactory theory for the mechanism of the photosynthetic process.

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JAMES CURRY
SAM F. TRELEASE

COLUMBIA UNIVERSITY

[2] O. Reitz and K. F. Bonhoeffer, *Z. physik. Chem.*, A, 172: 369–388, 1935.

BOOKS RECEIVED

BIGELOW, ROBERT P. *Directions for the Dissection of the Cat.* Revised edition. Pp. xi + 65. 5 figures. Macmillan. $0.90.

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