THE EFFECT OF RADIOCARBON ON THE RATE OF CARBON DIOXIDE UTILIZATION DURING PHOTOSYNTHESIS*

O. HOLM-HANSEN, V. MOSES, C. F. VAN SUMERE** AND M. CALVIN
Radiation Laboratory and Department of Chemistry, University of California, Berkeley, Calif. (U.S.A.)

An isotope effect is a difference, in either the rate or the equilibrium of a reaction, induced by the difference in mass between the isotope under consideration and the one which is used for comparison, usually the natural mixture. The existence of such an isotope effect, both in simple chemical reactions as well as in the more complex reaction sequences found in metabolic studies, is well established[1-5]. Occasionally, it is most convenient to measure the rate of a reaction by using isotopic material and to depend upon the characteristics of the isotope (*e.g.*, radioactivity) to provide the required sensitivity for the measurement. When the information desired is the absolute rate by which the reaction proceeds with the natural mixture of isotopes, it is necessary to apply a suitable correction factor to the rate thus measured; this will convert it from the rate of the reaction of the isotopic material to the rate of the reaction of the natural mixture of isotopes.

Thus, various investigators, studying the rate of photosynthesis, have found that carbon-14 is assimilated more slowly than carbon-12 by amounts varying from 6 % to 17 %[6-8]. Similar rate differences between carbon-14 and carbon-12 have been observed in single-step chemical reactions[9,10]. While the reaction rate is altered by the use of different isotopes, the nature of the reaction product, apart from isotopic composition, is not changed. In a biological system, the product is very often the result of a complex sequence of reactions which are related in several ways. If the isotopic difference is maintained throughout such a complex series of transformations, it is conceivable that the relationship between the starting material and the final products may be so different quantitatively as to appear as a qualitative difference. It is not to be expected, however, that the direction of an isotope effect in different reactions *will* always be the same, no matter what reaction is involved. Thus it is very unlikely that the situation of a one-sided isotope effect, carrying through a long sequence of reactions to produce a totally different metabolic pattern, will occur. It has been assumed, therefore, that the reactions of carbon-14 in biological systems will not be qualitatively different from those of carbon-12. Recently, however, BOICHENKO AND ZAKHAROVA[11,12] have reported that tracer amounts of carbon-14 (in which 0.08 % or more of the total carbon is present as carbon-14) reduced the rate of carbon dioxide uptake to 1/5th the control value (0.004 % carbon-14) for 10 min of photosynthesis. When 0.72 % of the total carbon was present as carbon-14, the rate was further

* The work described in this paper was sponsored by the United States Atomic Energy Commission.

** IRSIA Grant, 1957. Present address: Biochemical Department, Rijksuniversiteit Gent, Casinoplein 11, Ghent, Belgium.

References p. 591.

reduced to 1/40th of the control value. This implies that carbon-14 somehow interferes with the assimilation of carbon-12. Furthermore, the pattern of carbon-14 incorporation is reported to be altered to a marked degree as well. An effect like this is very different from the known isotope effect of carbon-14; as carbon dioxide containing 10% to 20% of $^{14}CO_2$ is commonly employed in our laboratory, these findings of BOICHENKO AND ZAKHAROVA prompted us to reinvestigate this point.

The experiments described below were designed to determine whether there is any effect on the rate of photosynthesis and the pattern of carbon dioxide incorporation in the presence of carbon dioxide containing 10% to 20% $^{14}CO_2$ when using either algae or a higher plant.

To test the effect of increasing concentrations of carbon-14 on the rates of photosynthesis and on the fixation patterns of carbon dioxide, *Chlorella pyrenoidosa* (1% suspension) was exposed, using techniques previously described[13], to labeled sodium bicarbonate of specific radioactivity ranging from 0.003 to 15 $\mu C/\mu mole$. The experiment reported in Table I was performed in a new apparatus[14] in which several

TABLE I

TOTAL INCORPORATION OF CARBON-14 BY *Chlorella* AFTER 2 MIN PHOTOSYNTHESIS IN THE PRESENCE OF $NaH^{14}CO_3$ OF VARYING SPECIFIC ACTIVITY

1.0 ml of cell suspension containing 10 μl of wet packed cells was aerated in the light (intensity 2,000 f.c.) with air containing 1% CO_2 for 25 min, immediately after which the air and CO_2 flow was stopped and 100 μl of 0.026 *M* $NaHCO_3$ (for specific activity see Table) were injected. The cells were allowed to carry on photosynthesis in the presence of $NaH^{14}CO_3$ for 2 min. The reaction was then stopped by the addition of 4 ml of boiling alcohol.

	Specific activity of added $NaHCO_3$ in $\mu C/\mu mole$	*$^{14}CO_2$ conc. as per cent of total CO_2*	*Total fixation of ^{14}C in counts/min*	*Ratio*	*Expected from spec. act.*	*Found fixed*
A	1.5	2.2	517,000	A/C	547	530
B	0.057	0.084	22,000	A/B	26.3	23.5
C	0.00274	0.00405	974	B/C	20.8	22.6

samples were shaken simultaneously over a light source. The radioactive bicarbonate solution, and later the boiling ethanol, were added without interrupting the shaking of the samples in order to ensure rapid and complete mixing. The results from the first experiment (Table I) show the total fixation of $^{14}CO_2$ by the cells after 2 min of photosynthesis, while in Fig. 1 is shown the fixation after varying times of exposure to $^{14}CO_2$ from 6 sec to 3 min. In these experiments the volume and molarity of the injected bicarbonate were constant. The data in Table I indicate that after 2 min there was no effect on the specific rate of carbon-14 fixation caused by varying the specific activity of carbon-14, while Fig. 1 indicates that the same holds true for exposure times of 6 sec to 3 min. In both of these experiments, the fluctuations in fixation from the theoretical values lie within the experimental error. The patterns of carbon-14 fixation into the alcohol-soluble compounds were determined in the 6-sec, 40-sec, 2-min, and 3-min samples of Fig. 1. No significant changes were seen in the distribution of carbon-14 among the various compounds in the samples supplied with varying specific activities of $^{14}CO_2$ for the times mentioned. The patterns for 3-min fixation time are shown in Fig. 2. These two experiments do not, however, indicate whether or not there is any change in the rate of total carbon dioxide utilization when

References p. 591.

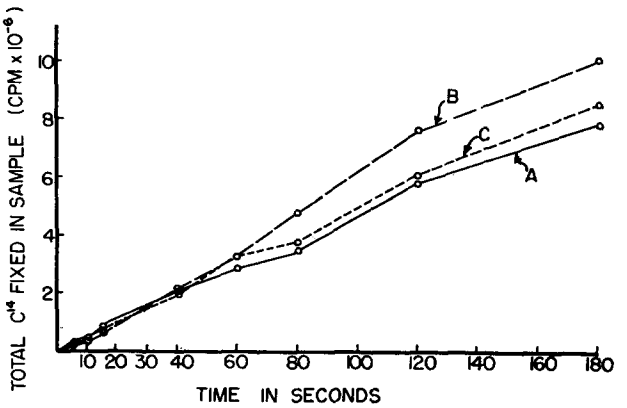

Fig. 1. Total incorporation of carbon-14 by *Chlorella* at various times in the presence of $NaH^{14}CO_3$ of varying specific activity. 3.0 ml of cell suspension containing 30 μl of wet packed cells were aerated in the light (intensity 7,000 f.c.) with air containing 1 % CO_2 for 10 min, immediately after which the air and CO_2 flow was stopped and 100 μl of 0.026 *M* $NaH^{14}CO_3$ were injected, the specific activity of which was 15 μC/μmole (curve A), 1.5 μC/μmole (curve B), and 0.15 μC/μmole (curve C). The cells were allowed to carry on photosynthesis in the presence of $NaH^{14}CO_3$ for times ranging from 6 sec to 3 min, the reaction then being stopped by the addition of 12 ml of boiling alcohol. The ordinates for curves B and C are multiplied by 10 and 100, respectively.

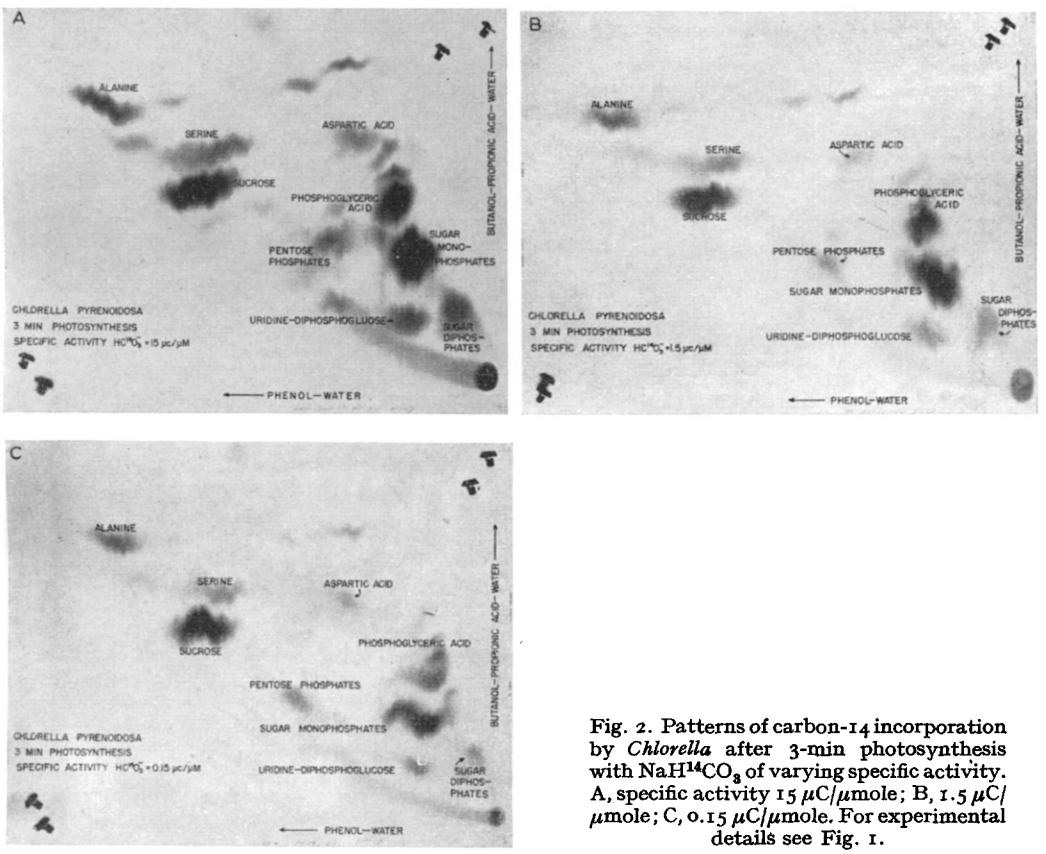

Fig. 2. Patterns of carbon-14 incorporation by *Chlorella* after 3-min photosynthesis with $NaH^{14}CO_3$ of varying specific activity. A, specific activity 15 μC/μmole; B, 1.5 μC/μmole; C, 0.15 μC/μmole. For experimental details see Fig. 1.

changing from an environment in which all the carbon is present as carbon-12 to an environment containing some carbon-14. To obtain an answer to this question, the rates of gas exchange of *Chlorella* during photosynthesis were measured in an apparatus[15,16] which automatically records the concentrations of oxygen, carbon dioxide, and radioactive carbon dioxide every 7 seconds. The rates of gas exchange were first determined with an atmosphere of air containing 0.79 % CO_2 being circulated through the suspension, and then again after the introduction of a known amount of $^{14}CO_2$ into the system. The amount of carbon-14 introduced was sufficient to make a a final isotopic concentration of about 5 % $^{14}CO_2$ of the total CO_2 present which was now raised to 0.90 %. The rates of absorbtion of CO_2 before and after the introduction of the carbon-14 were 21.5 and 20.5 arbitrary units of CO_2/min, while the corresponding oxygen evolution rates were 16 and 15 arbitrary units of O_2/min. It is thus seen that there was no significant effect on the photosynthetic rate when carbon-14 was introduced into the system. This same type of experiment was then performed with a leaf of a Primrose plant (*Primula* sp.), this being the plant genus used by BOICHENKO AND ZAKHAROVA[11]. Immediately upon removal of the leaf from the plant, the base of the petiole was submerged in water in a small test tube and then placed in the illumination chamber*. The rates of oxygen and carbon dioxide exchange before and after the introduction of carbon-14 (final $^{14}CO_2$ percentage about 5 % of total CO_2) were 2.95 and 2.98 arbitrary units of CO_2 absorbed per min and 2.50 and 2.90 arbitrary units of O_2 evolved per min, respectively. Once again it is seen that there was no significant change in the rate of gas exchange caused by the introduction of carbon-14. It was noticed that after the introduction of carbon-14 to both *Chlorella* and the Primrose leaf the rate of utilization of the $^{14}CO_2$ was a little slower than that of $^{12}CO_2$ (the infrared CO_2 analyzer detects mainly $^{12}CO_2$, and not $^{14}CO_2$; the rate of $^{14}CO_2$ utilization was determined by measuring the radioactivity in the circulating atmosphere using an ionization chamber). Quantitatively this isotope effect is within the range mentioned at the beginning of this communication, but the important observation is that the presence of the $^{14}CO_2$ did not have any appreciable effect on the absorption of the $^{12}CO_2$, or upon the specific rate or pattern of $^{14}CO_2$ utilization.

The above experiments, which are but a few done in our laboratory in an attempt to confirm the results of BOICHENKO AND ZAKHAROVA[11,12], unequivocally demonstrate that aside from the usual isotope effect there is no effect of carbon-14 which would invalidate its use as a tracer substance in biological systems at specific-activity levels as high as 20 % carbon-14, at least for short periods of time.

SUMMARY

In view of the wide application of radioactive carbon dioxide in tracer experiments together with a reported anomalous effect of the isotope content on the tracer behavior, we have investigated the effect of varying specific activity of the carbon dioxide on both the rate and pattern of photosynthetic carbon fixation. We have found no effect of the carbon-14 other than the ordinary mass effect in both algae and higher plants.

* The illumination vessel as depicted in reference 15 was replaced by a modified illumination chamber which is similar in size and shape, but suitable for working with leaves as one whole side can be removed.

References p. 591.

REFERENCES

1 J. W. WEIGL AND M. CALVIN, *J. Chem. Phys.*, 17 (1949) 210.
2 P. E. YANKWICH AND M. CALVIN, *J. Chem. Phys.*, 17 (1949) 109.
3 S. ARONOFF, *Techniques of Radiobiochemistry*, Iowa State College Press, Ames, Iowa, 1956, pp. 1–15.
4 D. A. SEMENOW AND J. D. ROBERTS, *J. Chem. Educ.*, 33 (1956) 2.
5 D. L. BUCHANAN, A. NAKAO AND G. EDWARDS, *Science*, 117 (1953) 541.
6 J. W. WEIGL, P. M. WARRINGTON AND M. CALVIN, *J. Am. Chem. Soc.*, 73 (1951) 5058.
7 R. W. VAN NORMAN AND A. H. BROWN, *Plant Physiol.*, 27 (1952) 691.
8 E. STEEMAN NIELSEN, Extrait du *J. Conseil, permanent intern. exploration mer*, 18 (1952) 117.
9 J. BIGELEISEN AND L. FRIEDMAN, *J. Chem. Phys.*, 17 (1949) 998.
10 G. A. ROPP AND V. F. RAAEN, *J. Am. Chem. Soc.*, 74 (1952) 4492.
11 E. A. BOICHENKO AND N. I. ZAKHAROVA, *Conference of the Academy of Sciences of the USSR on the Peaceful Uses of Atomic Energy*, July 1–5, 1955; pp. 121–125.
12 E. A. BOICHENKO AND N. I. ZAKHAROVA, *Biochemistry (USSR)*, 21 (1956) 377.
13 V. MOSES, O. HOLM-HANSEN AND M. CALVIN, *Biochim. Biophys. Acta*, 28 (1958) 62.
14 V. MOSES, *University of California Radiation Laboratory Chemistry Division Quarterly Report*, December 1957.
15 A. T. WILSON AND M. CALVIN, *J. Am. Chem. Soc.*, 77 (1955) 5948.
16 J. A. BASSHAM, K. SHIBATA AND M. CALVIN, *Biochim. Biophys. Acta*, 17 (1955) 332.

Received December 2nd, 1957

THE METABOLISM OF CORTISOL AND PROGESTERONE BY CULTURED UTERINE FIBROBLASTS, STRAIN U12-705*,**

MAX L. SWEAT, BERNARD I. GROSSER, DAVID L. BERLINER, H. EARLE SWIM, CHARLES J. NABORS, JR., AND THOMAS F. DOUGHERTY

Department of Obstetrics and Gynecology, Department of Anatomy of Utah College of Medicine, Salt Lake City, Utah, and Department of Microbiology of Western Reserve University Medical School, Cleveland, Ohio (U.S.A.)

Steroid metabolism of the liver has been studied extensively since ZONDEC[1] first observed the liver to have the capacity of inactivating steroid hormones. It has been only recently, however, that evidence pertaining to the metabolism of steroids by extrahepatic tissues have appeared in the literature[2,3]. In this report data are presented which demonstrate that human fibroblasts propagated *in vitro* convert cortisol and progesterone to a wide variety of steroid products.

METHODS

Stock cultures of human fibroblasts, strain U12-705, were propagated in medium 705 (5% chick embryo extract, 20% normal horse serum, 75% solution 703[4]) as described by SWIM AND PARKER[5]. Experiments were conducted in 16 × 150 mm tubes containing 50,000–120,000 cells in 6 ml of medium supplemented with ^{14}C-steroid (8,000–10,000 counts/min, specific activity cortisol-4-^{14}C $3.2 \cdot 10^6$ counts/min/mg, progesterone-4-^{14}C, $1.07 \cdot 10^7$ counts/min/mg). The tubes were inclined at

* A preliminary report of this paper was read at the Forty-Eighth Annual Meeting of the American Society of Biological Chemists, *Federation Proc.*, 16 (1957) 258.

** Aided by U.S. Public Health Grants 4207-(C) and E-1547.

References p. 595/596.