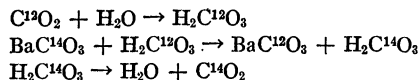


Exchange of Carbon Dioxide Between Barium Carbonate and the Atmosphere¹

W. D. ARMSTRONG and JACK SCHUBERT

Department of Physiological Chemistry and Laboratory of Dental Research, University of Minnesota

It was observed that precipitates of barium carbonate ($\text{BaC}^{14}\text{O}_3 + \text{BaC}^{12}\text{O}_3$) used as standards for radiocarbon (C^{14}) measurements appeared to lose a significant amount of radioactivity when stored for several weeks in contact with air in covered Petri dishes. In an experiment designed to test these observations and in which the Geiger-Müller counts were referred to a uranium standard a sample of radioactive barium carbonate lost 2.7 per cent of its original activity in 6 days. These findings could be explained as being the result of the incorporation in the precipitate of C^{12}O_2 from the atmosphere in exchange for C^{14}O_2 . A mechanism for the exchange was postulated as follows:



Evidence that the exchange occurs and that the hypothesized mechanism is correct was obtained when it was demonstrated that (a) no loss of radioactivity by barium carbonate occurs in the absence of water vapor or carbon dioxide, and (b) radioactive carbon dioxide is released to the atmosphere, when the exchange occurs, in an amount equivalent to the decrease in radioactivity of the barium carbonate.

admitted in sufficient amount to restore the pressure to atmospheric after the desiccator had been exhausted with a water pump. After the times indicated, the carbon dioxide present as a gas in the desiccator was collected in sodium hydroxide solution and the carbonate of the whole of this solution, or of an aliquot, precipitated as barium carbonate (1). The radioactivity of these precipitates and of the original barium carbonate, after drying and reweighing, were then determined. The activity of all samples was corrected for changes in counter performance by reference to counts of a uranium standard which was counted at least four times alternately with each sample.

The total activity of the precipitates, all of which were "thick" samples, were calculated by multiplying the observed count, corrected for counter performance and background, by the ratio of the thickness of the precipitate (expressed as mg./cm.²) to 20. The relatively large statistical error shown for the radiocarbon found in the desiccator gas in Experiment 1 is due to the fact that the large amount of carbon dioxide necessitated counting a barium carbonate precipitate which contained only $\frac{1}{10}$ of the total carbon present in the gas phase of the desiccator.

In Experiment 1 the original precipitate lost 37.4 per cent of its radioactivity and, within the limits of experimental error, all of the radioactivity so lost was recovered as carbon dioxide in the atmosphere above the precipitate. The loss in weight of the precipitate after the treatment is probably within the experimental error of weighing the brass dishes and would account for no more than 1.74 per cent of the decrease in radioactivity. In other trials similar to Experiment 1 radioactivity equivalent to 12–48 per cent of the total was lost by the precipitates in 48–60 hours.² These results, obtained in the

TABLE 1
EFFECT OF WATER AND CARBON DIOXIDE ON LOSS OF RADIOCARBON BY BARIUM CARBONATE (t = 22° C.)

Exp. No.	Treatment		Original weight of precipitate (mg.)	Weight of precipitate after treatment (mg.)	Total activity of precipitate (counts/minute)*		Total activity recovered in CO_2 in desiccator*
	Conditions	Time (hr.)			Before treatment	After treatment	
1	$\text{H}_2\text{O} + \text{C}^{12}\text{O}_2$	65	178.1	175.0	1,782 ± 19	1,115 ± 16	620 ± 90
2	$\text{H}_2\text{O} + \text{N}_2$	71	176.3	176.3	1,710 ± 18	1,693 ± 18	B.G.†
3	$\text{P}_2\text{O}_5 + \text{C}^{12}\text{O}_2$	41	157.5	157.0	1,448 ± 16	1,460 ± 16	B.G.

* The activity results are shown ± the square root of the sum of the squares of the errors of the sample and background.

† B.G. = Not significantly different from the background count.

Precipitates of barium carbonate containing radiocarbon with the weights shown in Table 1 and surface areas of 5.73 cm.² were collected in brass, Buchner-type funnels, as previously described (1). After the radioactivity of the precipitates had been measured with a thin mica window counter, each funnel and precipitate was separately placed in a vacuum desiccator of 2-l. capacity under the conditions indicated in Table 1. In Experiments 1 and 2 the bottom of the desiccator was covered with a layer of acidulated water in order to saturate the atmosphere of the container with water vapor. In Experiment 3 the air in contact with the barium carbonate was kept dry by a layer of phosphorus pentoxide covering the bottom of the desiccator. The gases (C^{12}O_2 or N_2) were

presence of water vapor and carbon dioxide, and the fact that no loss of activity occurs when barium carbonate is treated as indicated in Experiments 2 and 3, are evidence that the mechanism postulated above is correct. Under the conditions of these experiments it appears that the exchange, as in Experiment 1, occurs uniformly throughout the precipitate, since thorough mechanical mixing of the barium carbonate after

² A sample of enriched C^{13} barium carbonate containing 2.86 atom per cent excess of C^{13} was subjected to similar treatment. The atom per cent excess of C^{13} was reduced to 2.64, showing that a definite exchange with atmospheric CO_2 had occurred. Since the C^{13}O_2 released was diluted 75-fold, it was not possible to ascertain with accuracy the resulting increase in $\text{C}^{13}/\text{C}^{12}$ ratio in the atmosphere of the desiccator, although qualitatively this gas showed an enriched C^{13} content above its original composition. The enriched BaCO_3 and the analyses were kindly provided by Alfred O. Nier, Department of Physics, and Nathan Lifson, Department of Physiology, University of Minnesota.

¹ This work was carried out with the aid of grants from the John and Mary R. Markle Foundation and the Graduate School of the University of Minnesota.

the treatment caused no change in activity. This would not have been the case had the exchange occurred only on the surface layers of the precipitate.

These observations indicate the necessity for preserving carbonates which, in the solid state, are to be used as isotopic carbon reference materials in such manner that carbon dioxide or water, or both, are excluded from contact with the solid substance. Further, the observations confirm the possibility that one of the paths by which sunflower leaves absorb carbon dioxide from the air is by exchange of carbon dioxide with insoluble carbonates in the leaves (2).

References

1. ARMSTRONG, W. D., and SCHUBERT, J. *Anal. Chem.*, in press.
2. SMITH, J. H. C., and COWIE, D. B. *Plant Physiol.*, 1941, 16, 257.

The Use of Isotopes to Determine the Rate of a Biochemical Reaction¹

HERMAN BRANSON

*Department of Physics,
Howard University, Washington, D. C.*

Radioactive and stable isotopes are being used increasingly to determine the rates of biochemical reactions for which suitable techniques were previously not available. The theory of this application of isotopes has been discussed by Zilversmit and his collaborators (2) and by Branson (1). Both formulations show that in order to determine the rate for a substance, B, formed from A, the experimenter must have information on the time dependence of A and B.

Branson's integral equation formulation gave

$$M(t) = M(0)F(t) + \int_0^t R(\theta)F(t - \theta) d\theta,$$

where $M(t)$ is the amount of substance present at time t ; $M(0)$ is the amount initially present; $F(t)$ is the "metabolizing" function—the function which multiplies the original amount to give the amount present at time t ; and $R(t)$ is the rate of accumulation. The equations applicable to the system $A \rightarrow B$, where A is transformed into B by a first order chemical reaction, are:

$$\begin{aligned} A^*(t) &= \int_0^t R(\theta)F(t - \theta) d\theta \\ B(0) &= B(0)F_1(t) + \int_0^t R_1(\theta)F_1(t - \theta) d\theta \\ B^*(t) &= C \int_0^t A^*(\theta)F_1(t - \theta) d\theta, \end{aligned} \quad (1)$$

which assume that the amount of B present is constant and $R = CA$. The starred quantities refer to the substances isotopically tagged. Hence, if we are interested in the R and F associated with $B(t)$, we must determine experimentally $A^*(t)$, $B^*(t)$, and $B(0)$.

¹ This work is being supported in part by a grant from the Research Corporation of New York.

The procedure is open to severe criticism in most complex biological systems, for it requires the unequivocal proof that B is the exclusive product of A. In general that will not be true; and, if it were, the scheme would be difficult to establish. Under the dynamic conditions existing in biological systems, we can expect side reactions and complex chains, some of which may lead eventually from A to B.

One procedure for eliminating the dependence upon measurements of $A^*(t)$ would be to introduce the tagged substance suspected as being the precursor of $B^*(t)$ in one system or series of animals and the tagged substance, $\bar{B}(t)$, in a similar system. From the first system we have

$$B^*(t) = \int_0^t R(\theta)F(t - \theta) d\theta \quad (2)$$

and from the second,

$$\bar{B}(t) = \bar{B}(0) F(t), \quad (3)$$

which are sufficient to determine $F(t)$ and $R(t)$ without dependence upon precursors of B.

Although of value, the preceding technique is probably as vulnerable as the first, since the measurements are made upon different systems. The experimenter would have greater confidence in his rate determinations if they were based solely upon measurements of the substance under study and limited to simultaneous measurements in a single system.

These desired conditions may be obtained by the use of a doubly-tagged² substance in a single system. We may introduce the suspected tagged precursor of B and follow experimentally the level of $B^*(t)$. At the same time, we may inject some $\bar{B}$ —that is, a small amount of the chemically similar substance but tagged by the use of a different radioactive isotope or by a rare stable isotope. Thus, Equations 2 and 3 permit the simultaneous determination of $R(t)$ and $F(t)$ in a single system by measurements of B alone.

The number of possible combinations of isotopes available depends in large measure upon the problem and the skill of the experimenter. Under all circumstances, radioactive and stable pairs such as (H^2 , H^3), (C^{13} , C^{14}), (S^{34} , S^{36}) and the radioactive pair (Fe^{55} , Fe^{59}) can be used for these elements. No appropriate radioactive member exists for oxygen or nitrogen; however, in many experiments they can be coupled with hydrogen, carbon, or sulfur. The assays will require the simultaneous use of the mass spectrometer and the Geiger-Müller counter, but such equipment is now rather widely available.

Experiments of this type are being planned for our laboratory using a small 60° Nier Type spectrometer and conventional Geiger-Müller equipment.

References

1. BRANSON, HERMAN. *Bull. Math. Biophys.*, 1946, 8, 159-165; 1947, 9, 93-98.
2. ZILVERSMIT, D. B., ENTEMAN, C., and FISHLER, M. C. *J. gen. Physiol.*, 1943, 26, 325-331; ZILVERSMIT, D. B., ENTEMAN, C., FISHLER, M. C., and CHAIKOFF, I. L. *J. gen. Physiol.*, 1943, 26, 333-340.

² "Doubly-tagged" designates a substance, part of whose molecules are tagged by one isotope and part by another; e.g. methionine, with some molecules having S^{34} and some S^{36} , or betaine, with some molecules having N^{14} and some C^{13} .