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*).

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The Use of Isotopes to Determine the Rate of a Biochemical Reaction[1]

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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:

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

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).

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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, $\overline{B}(t)$, in a similar system. From the first system we have

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

and from the second,

$$\overline{B}(t) = \overline{B}(0)\ F(t), \qquad (3)$$

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-tagged[2] 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 $\overline{B}$—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^{35}) 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

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[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^{35}, or betaine, with some molecules having N^{15} and some C^{13}.