IN THE LABORATORY

An Extension of the Isotope Dilution Method

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The principle of isotope dilution forms the basis of a method of quantitative analysis for mixtures which contain components of similar properties and which are therefore difficult to resolve by conventional methods (*2*). An isotopic analogue of the compound to be determined is added in known quantity and subsequently reisolated from the mixture. The decrease in isotope concentration of the labeled carrier caused by admixture with the normal constituent will be a measure of the quantity of normal compound originally present.

A method which may be described as reverse isotope dilution has been employed in the study of various problems of intermediary metabolism. In order to establish the origin of a metabolite from a labeled precursor on the basis of its isotope content, it is necessary to isolate the metabolite in pure form. When the amounts of metabolite present are too small to permit direct isolation, normal carrier may be added to facilitate recovery provided the metabolite originally contained a sufficiently high isotope concentration. Since both the amount and the isotope concentration of the metabolite are unknown, this procedure yields only the qualitative answer as to whether or not an excess of isotope is present. It is possible, however, to estimate more precisely the original isotope concentration of the metabolite if separate isotope dilution experiments are carried out with two aliquots of the metabolite solution. If the quantities of normal carrier added in each case are different, the two samples of reisolated material will contain different isotope concentrations. The following two equations will apply:[1]

$$y = \left(\frac{c_x}{c_y} - 1\right) x' \qquad (1)$$

$$z = \left(\frac{c_x}{c_z} - 1\right) x'' \qquad (2)$$

$$x' + x'' = x; \quad x'' = ax'.$$

In the above, x = mols of metabolite, x', x'' = mols of metabolite in aliquot, y, z = mols of normal carrier, c_x = isotope concentration of metabolite, and c_y, c_z = isotope concentration of mixture of reisolated carrier plus metabolite. Solving for c_x and x,

$$c_x = \frac{(ay - z)\, c_y c_z}{ayc_y - z \cdot c_z} \qquad (3)$$

$$x = \frac{\left(1 + \frac{1}{a}\right)(ayc_y - zc_z)}{c_z - c_y} \qquad (4)$$

If the experimental conditions are so chosen that $a = 1$ and $y = 2z$, equations (3) and (4) become

$$c_x = \frac{c_y c_z}{2c_y - c_z} \qquad (5)$$

$$x = \frac{2_z(2c_y - c_z)}{c_y - c_z} \qquad (6)$$

Since the values for c_x and x are a function of $2c_y - c_z$, *i.e.* the relatively small difference between two similar quantities, it is evident that the accuracy of this method of analysis will be determined by the precision attainable in the isotope analysis of c_y and c_z. If, for example, y and z are 10 and 20 times x, and if the error in the determination of c_y and c_z is 1%, the probable error for c_x will be 20%. An error of this order is acceptable for most metabolic experiments in which the range of biological variation is of similar magnitude.

[1] Since in the type of experiment under consideration the isotope concentration of the metabolite will not be high enough to change its molecular weight significantly, the simplified relation for isotope dilution can be employed (see *1*).

References

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Assay of Bacitracin in Body Fluids

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Since the published methods (*1, 2*) for the assay of bacitracin are not sensitive enough to detect small quantities of the antibiotic in body fluids, it seemed desirable to develop a procedure to meet these requirements. The following procedure, which will detect as little as 0.02 units/cc of bacitracin, has been used successfully with blood serum, urine, and saliva.

The culture used is a hemolytic streptococcus, Upjohn 24–5, and is the Shwartzman strain received by us in March 1935. This culture is maintained by weekly transfers on blood agar. Daily transfers are made in brain-heart infusion broth.

Preparation of plates. Add 20 cc of F.D.A. Penicillin assay agar to each Petri dish and allow to harden. These plates may be stored in the refrigerator for several days. Immediately before using the plates, add 4 cc of Meat