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

Infusion Agar which has been inoculated with 5 cc of an 18-hr culture of streptococci/100 cc of agar. Place 6 penicylinders at equidistance on each plate.

Preparation of standard. Six solutions of the standard are prepared in buffer pH 6.0 at concentrations of 1.0, 0.1, 0.08, 0.06, 0.04, and 0.02 units/cc.

Preparation of sample. Body fluids may be run undiluted, or any dilution necessary may be made in pH 6.0 buffer.

Method of assay. Eight plates are prepared for the determination of the standard curve. Each plate receives in the penicylinders 0.2 cc of each of the prepared standard solutions. Five plates are used for the test sample, each plate receiving 0.2 cc of two dilutions of the sample in duplicate. Likewise, each of the test plates receives 0.2 cc of each of the 0.1-unit and the 0.02-unit standard. All plates are incubated at 37° C for 18 hrs and the zone of inhibition measured.

Calculation. From the readings of the standard solution a curve is plotted. The readings of the test sample are plotted on this curve and the number of units calculated.

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An Apparatus to Facilitate Intravenous Injections in the Mouse[1]

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This paper describes a method of transillumination of the tail veins of mice and our results in using it for intravenous injections.

The equipment consists of the box shown in Fig. 1, a lamp, a hand lens, and a mouse holder fashioned from a Lucite base and an attached wire screen, shaped to hold the mouse. The mouse is permitted to run into the holder and is confined by a stopper with a hole at its base which allows the tail to protrude. The latter is conveniently fashioned from a one-hole rubber stopper. This holder is then placed on top of the box, which has clips to receive and keep it in place, as shown in Fig. 2. When the holder is in place, the tail is just over the slit that transmits the beam of light. This slit is about 1½″ long, and its width can be adjusted to the mean diameter of the tail by movable side slots. At its proximal end, the tail is held by a clip on the box, and the operator holds the tail at the distal end to keep it taut during the injection.

[1] This document is based on work performed under Contract No. W–31–109–eng–38 for the Manhattan Project at the Argonne National Laboratory, operated by the University of Chicago. The authors wish to acknowledge the helpful suggestions of William P. Norris, Thomas T. Tourlentes, and C. Phillip Miller.

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A curved bar of Lucite is used to transmit the light from its source to the tail. The curve is about 90°, and one end of the bar fits under the slit in the top of the box over which the tail is placed. The other end of the bar comes out of an opening on the side of the box. When a microscope lamp illuminates the Lucite end at the side of the box, the light is transmitted to the end under the slit in the box top, transilluminating the tail.

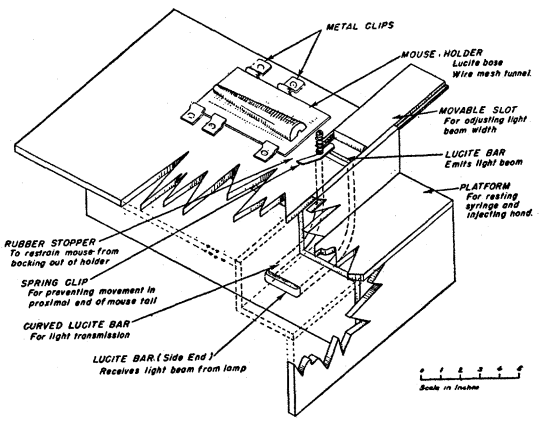

FIG. 1

The vein opposite the light is easily seen as a red cord. If the needle is introduced into the vein, the whole column of blood proximal to the needle will be replaced by the injectate. If, however, one penetrates either the fibrous band or through the vein into the core, injection is difficult and local blanching and subsequent swelling of the tail in this region is seen. There is a personal factor in

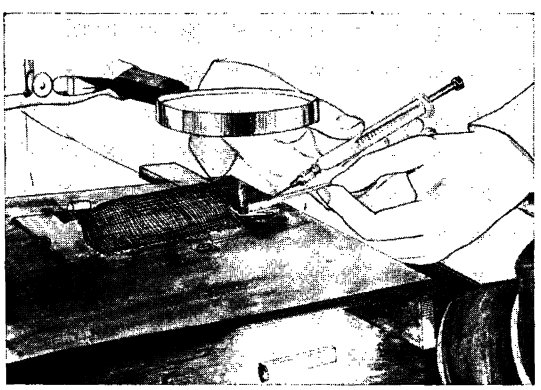

FIG. 2

acquiring the "feel" of keeping the needle superficial in order not to transfix the vein, but this can be overcome. We have found ½″ No. 27 needles most suitable.

In our experience it was possible with a few days practice to complete the injection of the tail veins of 90–95% of the animals. In a group of 170 we gave 22 injections/mouse over a period of 11 weeks. At the end of