The Potential of Radiometric Measurements in Physiology and Metabolism

The application of radiolabeled substrate for the measurement of bacterial metabolism is one of the most exquisite methods for the study of bacterial behavior. The specificity of the substrate method is high, and the sensitivity of the radiolabeled substrate is at least one order of magnitude greater than standard microbiological methods in current use. For example, one of the chemical differential points for identifying *Salmonella typhosa* from other gram-negative rods (e.g., *Escherichia coli*) is the lack of gas production (CO_2) from the fermentation of sugars, such as glucose, from *S. typhosa*. In the presence of carbon-14-labeled glucose, $^{14}CO_2$ is produced abundantly. The lesser sensitivity of the standard indicator methods for the determination of bacterial action on various substrates is partially responsible for some of the ambiguous results, i.e., production of gas (CO_2) may be variable or absent.

In this issue of the *Journal* Beckwith and Guidon describe a 5-hr radiometric serum antibacterial assay for gram-positive cocci (*1*). Their results compare very favorably with those obtained by the standard methods used to determine such assays. The advantages of the radiometric procedure are: (a) the lesser time required to obtain the results, (b) the completely objective data provided for interpretation, and (c) the minimization of technical time required. A similar approach to the determination of antibacterial assays has been reported by D'Antonio et al. (*2*), and they, too, found a good correlation between the standard method and the radiometric procedure.

It is unfortunate that the measurement of the metabolism of radionuclide-labeled substrates as an index of bacterial activity has not been pursued with greater determination. In 1972 DeBlanc et al. reported their experience with the automated radiometric measurement of antibiotic effect on bacterial growth by the metabolism of carbon-14-labeled glucose and evolution of $^{14}CO_2$ (*3*). Their results by the radiometric method were compared with those of the broth dilution method by constant factors characteristic of the organism and antibiotic tested. They found that the radiometric procedure was reproducible, quantitative, rapid, and sensitive to the inhibitory effects of antibiotics on bacterial growth. They concluded that the consistent relationship between the results of the radiometric and conventional techniques provided a basis for the development of an automated system for antibiotic susceptibility. In another report, the inhibition of glucose metabolism by antibiotics as an index of bacterial susceptibility was determined by the radiometric method (*4*). In this investigation the bacterial dose-response curves were defined. A relationship was found between the dose-response curves as determined by the inhibition of glucose metabolism and the minimal inhibitory concentrations of antibiotics determined by the serial broth dilution technique. In this study the concentration of antibiotic necessary to produce inhibition of growth depended on the site of antibiotic effect, i.e., interference with protein synthesis and genetic function, interference with cell wall structure and function, and interference with membrane function. Demonstration of the relationship between inhibition of glucose metabolism and the site of antibiotic effect documented the sensitivity of the radiometric method.

Strauss et al. compared the radiometric procedure with the conventional cystine tryptic agar sugar fermentation method for the identification of *Neisseria* species (*5*). The results between the two methods were identical except for the time required—3 hr for the radiometric method and overnight for the conventional method. Oxidase-positive *Diplococci* were examined by the conventional sugar method, fluorescent-antibody technique, and the radiolabeled substrate method. The radioisotope method appeared to be superior. These authors concluded that the radiometric method was more rapid and reliable than the other two. In a study of the fastidious organisms, Group D and *Viridans Streptococci* in blood, Beckwith found that all *Enterococci* were detected with appropriately radiolabeled media (*6*). Greater than 95% of the *Viridans Streptococci* were detected with radiolabeled substrates. These selected reports emphasize the advantages of radiolabeled substrates for diagnostic purposes in microbiology.

A very interesting, and possibly highly rewarding, application of the radiometric method is in the diagnosis of tuberculosis. Classically, culture techniques with Lowenstein-Jensen media re-

quire 6 wk for diagnosis. Middlebrook et al. using the radiometric method found that a medium composed of [^{14}C] palmitic acid, deficient in carbohydrates, and containing appropriate antimicrobial agents not active against the tubercle bacillus, was a practical substrate for this procedure (*7*). Detection of *M. tuberculosis* was significantly earlier by the radiometric method.

In addition to the direct application of radiometric methods to bacteriologic diagnoses, the technique has been used for other determinations using microorganisms indirectly. Chen et al. reported an assay method for the measurement of folic acid in human plasma and erythrocytes (*8*). Their assay was based upon the measurement of $^{14}CO_2$ produced from the metabolism of [1-^{14}C] gluconate by *Lactobacillus casei*. The $^{14}CO_2$ evolved was proportional to the amount of added DL-N-5-methyltetrahydrofolate. The correlation between the radionuclide and standard methods was very high. Investigators from the same institution described a radiometric microbiologic assay for the biologically active forms of niacin (*9*). In this procedure *Lactobacillus planatarum* produced $^{14}CO_2$ from L-[U-^{14}C] malic acid proportional to the quantity of niacin present. Again, correlation between the radiometric and standard methods was excellent.

Another very promising area is the application of the radiometric method for the measurement of the effect of hormones or drugs. For example, Singh et al. reported on the change in glucose metabolism of microorganisms by stimulation with triiodothyronine and thyroxine (*10*). We have observed similarly increased bacterial metabolism under T_3 and T_4 stimulation. Although this is only one experimental trial, the potential for evaluating other hormones under appropriate conditions is intriguing.

Cellular metabolism may also be measured by the radiometric method. Tran and Wagner found that the mitogens, PHA and Con A will stimulate lymphocyte carbohydrate metabolism (*11*). The increased lymphocytic activity was measured by the release of $^{14}CO_2$ from carbon-14-labeled glucose. The purpose of this study was to demonstrate that in vitro procedures can be used to measure lymphocyte immune response. It requires little imagination to extend this concept to the measurement of the effect of drugs on bacteria, normal cells, and neoplastic cells.

This short discussion, of course, does not cover the multitude of possible applications of radiometric measurement to many biologic situations, but barely touches the surface. These procedures for measuring physiologic and metabolic processes offer a high sensitivity and exquisite specificity for diagnostic purposes. Automated equipment makes the screening of large numbers of procedures a reality, providing the practical basis for extensive applications. The interested members in our field of applied radionuclides should actively pursue such fruitful endeavors.

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