RADIOMETRIC DETECTION OF THE METABOLIC ACTIVITY OF MYCOBACTERIUM TUBERCULOSIS

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A radiometric test capable of detecting the metabolic rate of M. tuberculosis within 18 hr after inoculation has been developed. The technique is based on the measurement of $^{14}CO_2$ produced by the bacterial metabolism of ^{14}C-U-glycerol or ^{14}C-U-acetate. The test is an important first step in the development of rapid radiometric techniques for clinical study of Mycobacterium tuberculosis.

Although more than 25 years have passed since the introduction of effective antibiotics, Mycobacterium tuberculosis is still an important public health problem. In vitro culture of this organism is still time-consuming and has hindered the development of techniques for rapid detection, identification, and testing for susceptibility to drugs.

In 1969, DeLand and Wagner developed a technique for automated detection of microbial metabolism by measuring the conversion of ^{14}C-labeled substrate (particularly ^{14}C-U-glucose) to $^{14}CO_2$ (*1*). Further work involved a comparison of standard and radiometric microbiologic techniques in blood cultures (*2,3*), radiometric detection of antibiotic effect on bacterial growth (*4*), automated detection of Hemophilus influenza (*5*), and immune inhibition of bacterial metabolism (*6*).

We have extended the principle of radiometric detection of microbial growth to the detection of the metabolism of M. tuberculosis, based on the measurement of $^{14}CO_2$ produced through the metabolism of ^{14}C-U-glycerol or ^{14}C-U-acetate in pure culture. These studies are an important preliminary step toward the development of more rapid techniques for the isolation of these organisms from clinical specimens and radiometric testing for their susceptibility to drugs.

MATERIALS AND METHODS

In the preliminary experiments, a fully susceptible strain and an isoniazid-resistant strain of M. tuberculosis were grown in Lowenstein–Jensen medium. The colonies were taken from the slants aseptically and suspended in saline to a final concentration of 2×10^8 organisms/ml, using a McFarland standard. The suspension was further diluted prior to use. The concentration of viable organisms at these dilutions was confirmed by routine plating on 7H10 agar plates (*7*).

The reaction system consisted of 20-ml multidose sterile vials containing 10 ml of agar-free and glycerol-free 7H10 medium, 1 μCi (0.1 ml) of ^{14}C-U-glycerol (Amersham/Searle), and the final suspension of M. tuberculosis (1.0 ml). Control

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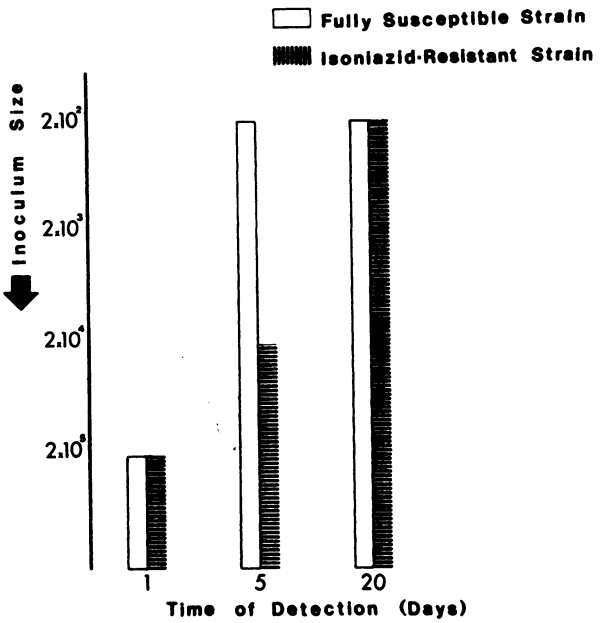

FIG. 1. Time of initial radiometric detection of M. tuberculosis in 7H10 medium as function of inoculum size.

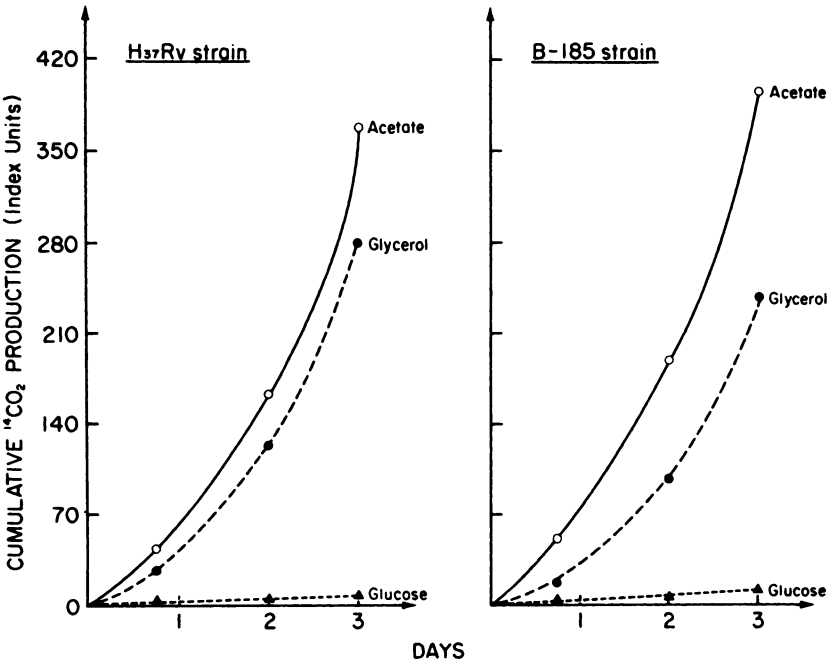

FIG. 2. Time of cumulative $^{14}CO_2$ production by metabolism of ^{14}C-labeled substrate for two strains of M. tuberculosis.

vials were prepared with no bacteria added. All vials were prepared in duplicate and incubated at 37°C. Measurements of bacterial metabolism were made with an ion chamber device (Bactecprototype, Johnson Laboratories, Cockeysville, Md.) at 1, 5, and 20 days. The measurement device flushed the $^{14}CO_2$-containing atmosphere of the experimental vials into the ion chamber and the radioactivity was determined automatically. Results were recorded on a scale of 0 to 100 as "index units" where 100 equaled 0.025 μCi ^{14}C activity. A reading of 20 or greater was considered as positive evidence of growth.

In a subsequent experiment, the rates of $^{14}CO_2$ production from ^{14}C-U-glucose, ^{14}C-U-glycerol, and ^{14}C-U-acetate (Amersham/Searle) were compared. Two fully susceptible strains of M. tuberculosis ($H_{37}Rv$, TMC #102 and B-185, a Johns Hopkins Hospital strain) were used and prepared as described, to a final concentration of 10^8 organisms/ml using a McFarland standard. The number of viable units was not determined. The reaction system now consisted of 5-ml multidose sterile vials which contained 0.6 ml of glycerol-free Middlebrook 7H9 medium with 0.1 ml of albumin–dextrose complex, ^{14}C-U-glycerol or ^{14}C-U-acetate, and 0.1 ml of the final suspension of M. tuberculosis (10^7 organisms/vial). Some experiments were done using 1 μl glucose but without the addition of the albumin–dextrose complex. All vials were prepared in duplicate and incubated at 37°C, including controls for background with autoclaved bacteria added. Measurements of bacterial metabolism were performed at 18, 48, and 72 hr. Details of the measurement device (Bactec R-301, Johnson Laboratories) have been described elsewhere (*8*). In all these experiments, sterility tests were performed on the positive samples and consisted of subculture in chocolate-agar and radiometric sterility testing with ^{14}C-U-glucose (*1–4*).

RESULTS

In the preliminary experiments, there was slower detection with low inoculum sizes, particularly with the isoniazid-resistant strain. However, the metabolism of 2×10^5 organisms initial inoculum was easily detected after 24 hr for both strains (Fig. 1). After 5 days all the inocula tested for the susceptible strain were detected, and after 20 days all the inocula for the isoniazid-resistant strain were also detectable (Fig. 1).

In the subsequent experiments, both strains metabolized ^{14}C-U-acetate to $^{14}CO_2$ more rapidly than ^{14}C-U-glycerol (Fig. 2). No significant $^{14}CO_2$ production was observed with ^{14}C-U-glucose. No $^{14}CO_2$ output was detected in the control vials and all sterility tests were negative.

DISCUSSION

The detection of M. tuberculosis in pure culture, although it may represent an important first step, does not imply that this method can be used in its present form for identification of these organisms in clinical specimens.

As few as 200 organisms initial inoculum could be detected after 5 or 20 days for the rapid (fully

susceptible) or slow growing (isoniazid-resistant) strains, respectively (Fig. 1). The data that very small inocula could be detected with ^{14}C-U-acetate (Fig. 2) suggest that the use of the latter or a more rapidly oxidizable ^{14}C-substrate will eventually lead to the detection of M. tuberculosis in clinical isolates.

The inability of M. tuberculosis to oxidize ^{14}C-U-glucose (Fig. 2) is fortunate, since this substrate can be used for the radiometric detection of contaminants in pure suspensions of tubercle bacilli. Most contaminating bacteria readily metabolize ^{14}C-U-glucose to $^{14}CO_2$ (*1–4*).

The existence of strains of M. tuberculosis resistant to one or more of the common drugs (isoniazid, streptomycin, para-aminosalicylic acid, ethambutol, rifampin) renewed the interest in the development of new drugs, with selection of therapy on the basis of susceptibility testing. Routine susceptibility testing of M. tuberculosis is difficult and time-consuming. The search for more rapid, reproducible, and sensitive methods continues. Since the radiometric method can be easily automated and the use of ^{14}C-U-acetate allows the detection of these organisms within 18 hr (Fig. 2), the development of a rapid testing of susceptibility to inhibition by drugs of M. tuberculosis seems to be feasible.

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