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RADIOMETRIC STUDIES OF MYCOBACTERIUM TUBERCULOSIS

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ABSTRACT

An in vitro assay system that included automated radiometric quantification of $^{14}CO_2$ released as a result of oxidation of ^{14}C- substrates was applied for studying the metabolic activity of **M. tuberculosis** under various experimental conditions. These experiments included the study of a) mtabolic pathways, b) detection times for various inoculum sizes, c) effect of filtration on reproducibility of results, d) influence of stress environment e) minimal inhibitory concentrations for isoniazid, streptomycin, ethambutol and rifampin, and f) generation times of **M. tuberculosis** and **M. bovis**. These organisms were found to metabolize ^{14}C-formate, (U-^{14}C) acetate, (U-^{14}C) glycerol, (1-^{14}C) palmitic acid, 1-^{14}C) lauric acid, (U-^{14}C) L-malic acid, (U-^{14}C) D-glucose, and (1-^{14}C) D-glucose, but not (1-^{14}C) L-glucose, (U-^{14}C) glycine, or (U-^{14}C) pyruvate to $^{14}CO_2$. By using either ^{14}C-formate, (1-^{14}C) palmitic acid, or (1-^{14}C) lauric acid, 10^7 organisms/vial could be detected within 24-48 hours and as few as 10 organisms/vial within 16-20 days. Reproducible results could be obtained without filtering the bacterial suspension, provided that the organisms were grown in liquid 7H9 medium with 0.05% polysorbate 80 and homogenized prior to the study. Drugs that block protein synthesis were found to have lower minimal inhibitory concentrations with the radiometric method when compared to the conventional agar dilution method. The mean generation time obtained for **M. bovis** and different strains of **M. tuberculosis** with various substrates was 9 ± 1 hours.

KEY WORDS: Mycobacterium tuberculosis; ^{14}C-substrates; Radiometric system; Drug-action; Generation time.

INTRODUCTION

In 1969 a radiometric technique was applied for measuring the metabolic activity of common clinical pathogens [12]. Since then several studies comparing standard and radiometric techniques in blood cultures and detection of drug effect on bacterial growth have been published [10,12,13]. The same technique has been extended to study the metabolic activities of **M. lapraemurium** [4] and **M. tuberculosis** [9]. These studies were initially aimed to the assessment of the metabolic pathways of these organisms. The next steps consisted of the study of the effect of drugs on **M. lepraemurium** [5] and on **M. tuberculosis** [6,14]. At the same time, several other experiments were carried out to better understand metabolic requirements and the influence of physicol and biochemical factors

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that alter the metabolism of both **M. lepraemurium** [7] and **M. tuberculosis.**

The present paper reports the results of these experiments with **M. tuberculosis.**

MATERIALS AND METHODS

Preparation of bacilli: Two strains of **M. tuberculosis** were obtained from the Trudeau Mycobacterial Culture Collection ($H_{37}Rv$ TMC n° 102, fully susceptible and $H_{37}Rv$ TMC n.° 303, resistant to 5 ug of isoniazid). One strain of **M. tuberculosis** (Erdman, TMC n.° 107, fully susceptible)and one strain of **M. bovis** (BCG, resistant to 5 ug of isoniazid) were obtained from Dr. Gardner Middlebrook, University of Maryland Hospital. Eighteen clinical isolates of **M. tuberculosis** were obtained from The Johns Hopkins Hospital Mycobacteriology Laboratory.

In initial experiments, bacteria were scraped from Lowenstein Jensen (L-J) slants and suspended in saline using a vortex mixer to disperse the organisms. In subsequent experiments, bacteria were grown in 50 ml of liquid 7H9 medium with 10% albumin dextrose complex (ADC) enrichment and 0.05% polysorbate 80 (Tween 80) in 250 ml erlenmeyer flasks. Colonies were taken from L-J slants and transferred to the liquid growth medium where they were grown for 6-8 days. The bacteria were then homogenized with a Sorvall Omni-Mixer at 8.5 speed scale twice for thirty seconds. When studying the effect of filtration, part of the suspension was passed through a prefilter (Millipore Corporation) at this point. The number of bacteria was estimated with MacFarland barium sulfate standards [1]. Serial dilutions of the suspension were performed, and the concentration of viable organisms was confirmed by routine plating on 7H10 agar [15].

Media: 7H9 with 10% ADC enrichment, K-36 buffer [21] and water were used in these experiments.

Reaction system: The reaction system for detection of $^{14}CO_2$ employed sterile 5 ml multidose serum vials (A.H. Thomas Co.). Each vial had a total volume of 1.0 ml to 1.5 ml and 1.0 to 5.0 uCi of ^{14}C-substrate along with 0.1 ml to 0.5 ml of bacterial suspension. All vials were prepared at least in duplicate. Control vials were prepared in the same way but with autoclave bacteria added. When studying the effect of drugs on the bacterial metabolism, extra controls with live bacteria without the drug were always prepared for comparison.

Radiometric measurement: The vials were incubated at 37°C. An ion chamber device (Bactec R-301, Johnston Laboratories) with a logarithmic scale up to 3,000 units was used to measure the bacterial metabolism. Details of the operation of the measurement device have been published previously [4]. The vials were sampled daily for at least 3 days in most of the experiments or up to 15 days when studying detection times. The results were expressed as "index units" (100 units = 0.025 uCi of ^{14}C activity) or as percent of the activity of control vials. Background readings ranged from zero to 8 index units. All readings above 20 index units were considered as positive for growth.

Contamination monitoring: This was performed on all positive samples and consisted of subculture on chocolate-agar plates and radiometric sterility testing with (U-^{14}C) glucose [10,12].

EXPERIMENTAL

Metabolic Pathways: The $H_{37}Rv$ fully susceptible strain of **M. tuberculosis** was used in 7H9 medium with ADC, at a final concentration of 1-3 x 10^8 organisms/ml. The reaction system consisted of 0.7 ml 7H9, 0.1 ml ADC, 0.1 ml of bacterial suspension (1 x 10^7/vial) along with 0.1 ml (1.0 uCi) of one of the following substrates (Amersham Corporation): ^{14}C-formate, (U-^{14}C) acetate, (U-^{14}C) glycerol, (U-^{14}C) glycine, (U^{14}C) L-malic acid, (U-^{14}C) pyruvate, (U-^{14}C) D-glucose, (1-^{14}C) D-glucose and (1-^{14}C) L-glucose. When using the carbon-14 labeled glucoses, ADC was omitted in both the growth medium and experimental vials. All vials were sampled daily for 3 days.

The highest $^{14}CO_2$ output was observed with ^{14}C-formate (Table I). Essentially, no oxidation was found with pyruvate, L-glucose or glycine.

TABLE I

Oxidation rates of ^{14}C-substrates by **M. tuberculosis** $H_{37}Rv$ (TMC 102) in 7H9 medium

	With ADC	
	Index Units *	% Formate
^{14}C-Formate	2.311±119	100±5
(U-^{14}C) Acetate	1,165±45	50±2
(U-^{14}C) Glycerol	628±16	27±1
(1-^{14}C) L-Malic acid	190±17	8±1
(U-^{14}C) Pyruvate	10±4	0.4±0
(U-^{14}C) Glycine	3±1	0.1±0
	Without ADC	
	Index Units *	% Formate
^{14}C-Formate	244±12	100±5
(U-^{14}C) D-Glucose	26±4	11±2
(1-^{14}C) D-Glucose	23±2	9±1
(1-^{14}C) L-Glucose	0	0

* 100 index units = 0.025 uCi of ^{14}C-activity

Detection Times: The $H_{37}Rv$ TMC n.° 102 strain of **M. tuberculosis** was used in 7H9 medium with ADC at final concentrations ranging from 1.0 x 10^1 to 1.0 x 10^7 organisms/vial along with 2.0 uCi of substrate (Amersham Corporation): ^{14}C-formate, (1-^{14}C) palmitic acid or (1-^{14}C) lauric acid. Both palmitic and lauric acids were dissolved in bovine serum albumin (BSA) and catalase [16]. The vials were sampled at convenient intervals ranging from only 3 days up to 21 days with the smaller inocula.

In all experients, 10^7 organisms/vial could be detected within 24 hours. All ^{14}C-substrates were similar for inocula in the range of 10^7 to 10^5 organisms/vial, but (1-^{14}C) palmitic and (1-^{14}C) lauric acids were more effective than ^{14}C-formate for smaller inocula (Figs. 1 and 2).

Effect of Filtration: The Erdman strain of **M. tuberculosis** was used in 7H9 medium with ADC. After homogenization the suspension was divided into two parts: one was passed through a 47 mm diameter prefilter (Millipore Corporation) and diluted to yield final concentrations from 1 x 10^7 to 5 x 10^7 organisms/vial (filtered suspension); the remaining suspension was just diluted to yield the same concentrations (unfiltered suspension). The radioactive substrate was 1.0 uCi of ^{14}C-formate.

It was assumed previously that filtered samples would give more reproducible results because of the homogeneity of the suspensions and absence of clumps. However, as shown in Figure 3 no important difference was found between filtered and unfiltered suspensions in terms of standard deviation of the $^{14}CO_2$ production over 3 days detection. Similar results were found with the $H_{37}Rv$ TMC n.° 102 strain.

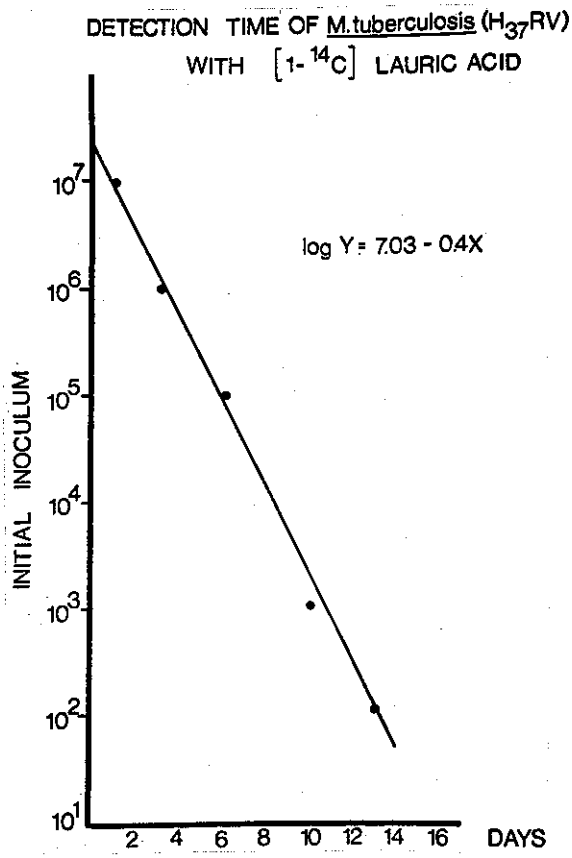

Fig. 1 — Detection times of **M. tuberculosis** ($H_{37}RV$ TMC 102) inocula with (1-^{14}C) lauric acid.

Stress Environment: The $H_{37}Rv$ fully susceptible strain of **M. tuberculosis** TMC n.° 102 was used in K-36 buffer and in water at final concentrations of 3 x 10^7 and 5 x 10^7 organisms/vial. Five microcuries of ^{14}C-formate or (1-^{14}C) lauric acid per vial were used.

As shown in Figure 4, the more adverse the environment the higher the $^{14}CO_2$ production over the first 24 hours. Then, the $^{14}CO_2$ output fell of rapidly over the next 48 hours.

Effect of Drugs: Eighteen clinical isolates of **M. tuberculosis** were obtained from the Johns Hopkins Hospital Mycobacteriology Laboratory, where they had been maintained on L-J agar slants for several months prior to study. The bacteria were suspended in 7H9

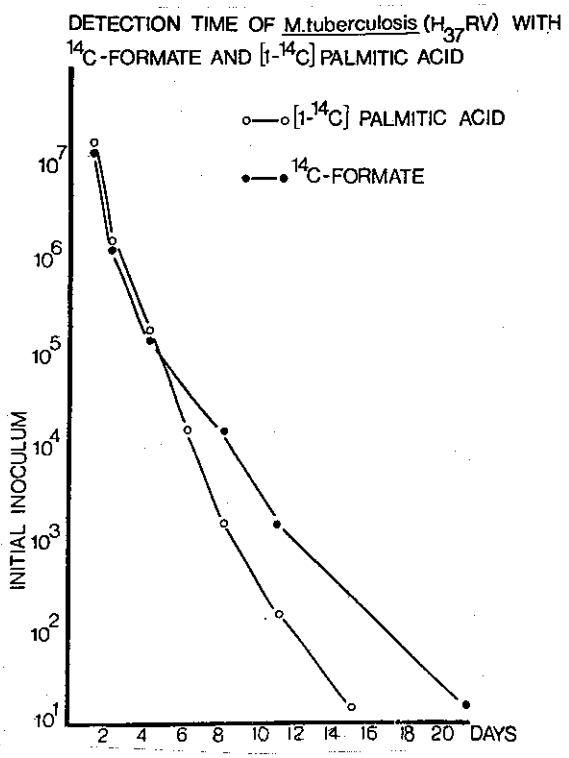

Fig. 2 — Detection times of **M. tuberculosis** (H_{37}Rv TMC 102) inocula with ^{14}C-formate and (1-^{14}C) palmitic acid.

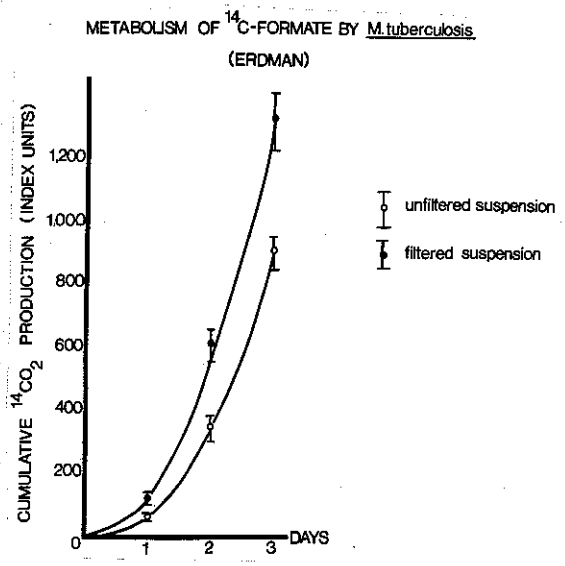

Fig. 3 — Comparison of the metabolism of ^{14}C-formate by filtered and unfiltered suspensions of **M. tuberculosis** Erdman

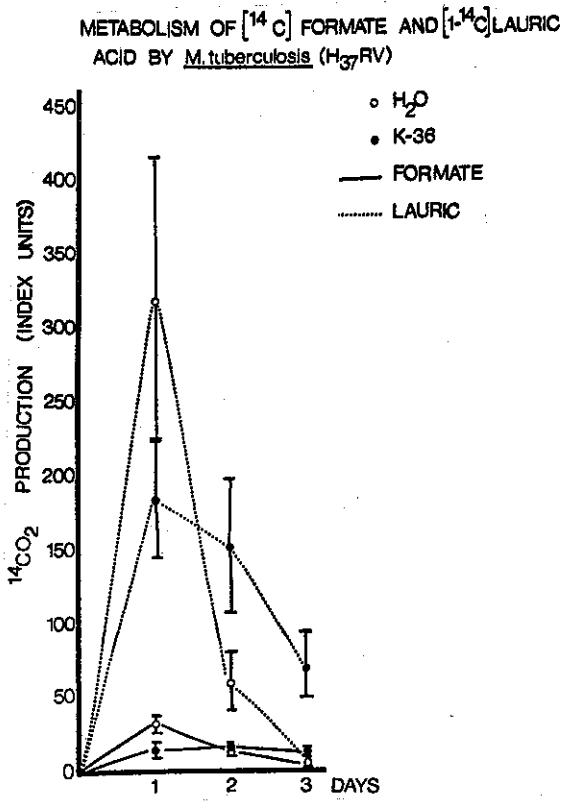

Fig. 4 — Metabolism of ^{14}C-formate and (1-^{14}C) lauric acid by **M. tuberculosis** (H_{37}RV TMC n.° 102) in water and K-36 buffer.

medium with ADC at a final concentration of 5 x 10^7 organisms/vial along with 1.0 uCi (0.1 ml) of ^{14}C-formate and 0.1 ml of drug solution. Drugs were prepared at a concentration of 1,000 ug/ml and stored at -20°C (maximum 30 days) until use. All drugs were diluted using sterile 7H9 medium so that the desired concentrations were delivered in 0.1 ml: isoniazid (0.1, 0.5, 1.0 and 5.0 ug/vial), streptomycin (1.0, 5.0 and 10.0 ug/vial), ethambutol (1.0, 5.0 and 10.0 ug/vial) and rifampin (0.05, 0.5 and 5.0 ug/vial). Vials with no drugs were prepared as controls. All vials were sampled daily for 3 days. Results were expressed as percent of $^{14}CO_2$ production of control vials at 48 hours. Dose-response curves were obtained and the radiometric minimal inhibitory concentration (MIC) estimated from the curves. Previous experiments [10,14] have shown that inhibition of 50% of the metabolic activity of the organisms correlated well with the routine MIC. Therefore, in this study the radiometric MIC was taken as that drug concentration that reduced the $^{14}CO_2$ output to 50% of that of control vials at 48 hours.

At the same time, 7H10 agar plates [15] containing oleic acid albumin-dextrose complex (OADC) and the same drug concentrations as above were prepared for correlation with the radiometric method. Figure 5 shows the dose-response curves for the drugs tested; Table II lists both radiometric and conventional agar dilution MIC for the same drugs. The radiometric MIC was lower than the conventional agar dilution MIC for all drugs, with the exception of ethambutol. This phenomenon has been reported with other organisms in the presence of drugs that block protein synthesis and genetic function [13].

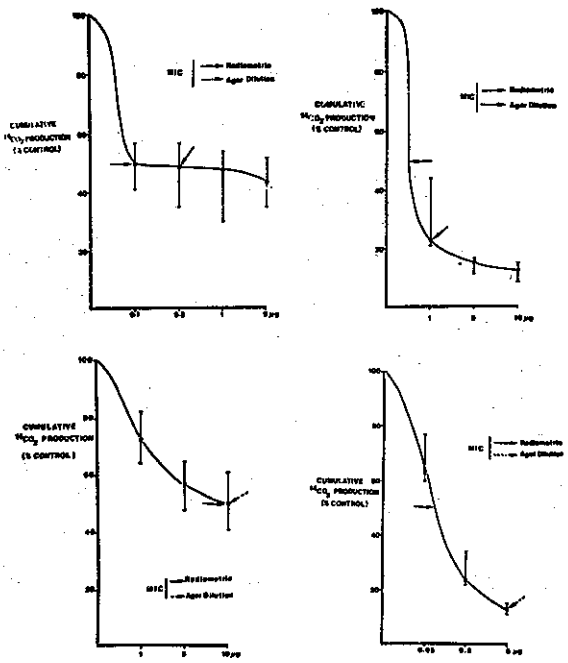

Fig. 5 — Dose — response curves of **M. tuberculosis** isolates to isoniazid (upper left), streptomycin (upper right), ethambutol (lower left) and rifampin (lower right).

T A B L E II
Minimal inhibitory concentrations (MIC)

Drug	Radiometric* (ug/ml)	Agar dilution (ug/ml)
Ethambutol	10	10
Isoniazid	0.10	0.50
Rifampin	0.14	5
Streptomycin	0.50	1

* In 18 clinical isolates of **M. tuberculosis**

Generation Time: The generation times were calculated from a) the logarithmic plots of the cumulative $^{14}CO_2$ production by the organisms, and b) the following equation:

$$G = \frac{0.301 \quad (t_2 - t_1)}{\log \Sigma C_2 - \log \Sigma C_1}$$

where ΣC_2 = cumulative $^{14}CO_2$ production at time 2, in "index units"; ΣC_1 = cumulative $^{14}CO_2$ production at time 1, in "index units"; $(t_2 - t_1)$ = time interval between ΣC_2 and ΣC_1 in hours [3].

Figure 6 shows the generation times obtained with ^{14}C-formate for **M. bovis** and **M. tuberculosis** strains ($H_{37}Rv$ TMC n.° 303, $H_{37}Rv$ TMC n.° 102 and Erdman). The overall mean generation time for all the organisms with various ^{14}C-substrates was 9 ± 1 hours.

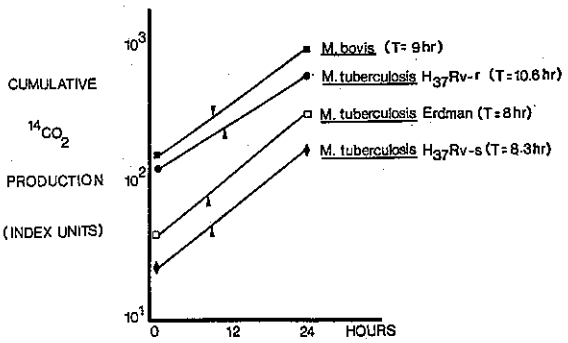

Fig. 6 — Generation times of **M. bovis** and **M. tuberculosis** strains as measured by the oxidation of ^{14}C-formate.

DISCUSSION

Despite the fact that only a small number of ^{14}C-substrates have been investigated, some conclusions on metabolic pathways can be drawn. The low oxidation rates found with (1-^{14}C) D-glucose and (U-^{14}C) D-glucose, which were also observed with **M. lepraemurim**, suggest that the hexose portion of the glycolytic pathway of **M. tuberculosis** $H_{37}Rv$ fully susceptible is predominantly anabolic (7,20). It is conceivable that in both organisms the oxidation of (U-^{14}C) glycerol may occur by a simple enzymatic action on glyceric acid rather than via the triose protion of the glycolytic pathway. However, a comparison of the oxidation rates of ^{14}C-formate and (U-^{14}C) glycerol shows that the latter is metabolized at a lower rate. This

supports the concept of multiple steps for glycerol oxidation rather than a simple enzymatic action on glyceric acid. In contrast to glycerol oxidation, the action of a formate dehydrogenase is probably the only step needed for formate oxidation [11]. Also, the non-oxidation of (U-^{14}C) glycine suggests that the oxidation of formate is an independent step from the glyoxylate and oxalyl-CoA pathway.

Because malic oxidation is poor as compared to formate, acetate and glycerol, a possible explanation is its transformation into pyruvate, a substrate poorly oxidized by **M. tuberculosis**. Finally, the best explanation for the oxidation of (U-^{14}C) acetate is via the acetyl-CoA and TCA cycle.

The detection times for small inocula using (1-^{14}C) palmitic acid, (1-^{14}C) lauric acid or ^{14}C-formate are in agreement with other authors [17]. However, the deleterious effect of high concentrations of ^{14}C-formate on the growth of **M. tuberculosis** in liquid medium, which has been reported by MIDDLEBROOK et al [17], was not observed, even in the presence of 5 uCi/ml with an inoculum size in the range of 10^7 organisms/vial. No significant difference was observed between (1-^{14}C) lauric and (1-^{14}C) palmitic acids. Although both substrates showed faster $^{14}CO_2$ production than ^{14}C-formate for small inoculum size, as low as 10 organisms could be detected with this substrate by 20 days.

In our initial experiments, when **M. tuberculosis** was grown on solid media, removal of clumps by filtration was essential, in order to obtain reproducible $^{14}CO_2$ outputs. However, the use of liquid 7H9 medium with 0.05% polysorbate 80 and homogenization eliminated sufficient amounts of clumps and gave reproducible results. Therefore, smaller, concentrations of organisms could be used to obtain inocula in the range of 10^7 organisms/ml without filtration. As more experience was gained with the homogenization procedure, the filtration step was no longer needed in our experiments.

When a microorganism is placed in a hostile environment, there is a tendency to overcome the adverses condition and therefore an incrase in respiration and $^{14}CO_2$ production occurs. This initial phenomenon is followed by a progressive decrease in the metabolism and death of the organism if the medium is not changed. When the medium is extremely hostile the organism usually dies immediately, but the transition from an environment where metabolism is still possible to another where metabolism is totally inhibited is difficult to establish [8]. This phenomenon was observed with **M. tuberculosis** both in K-36 buffer and in water. It is conceivable that the radiometric detection of **M. lepraemurium** in vitro [4] was feasible because of the early stimulatory effect of a hostile suspending medium such as K-36 buffer.

Over the last few years, several studies on radiometric detection, differentiation and drug susceptibility of mycobacteria have appeared in the literature [18,19,22]. The conclusion of YANGCO et al. [22] on susceptibility testing of **M. avium-intracelulare**, that the radiometric method is rapid, simple and needs further investigation is somewhat similar to the conclusions of one of our colleagues in 1978 [14]. In the present study we have determined not only the MICs, but also the dose-response curves. The MICs obtained for drugs that block protein synthesis were higher with the conventional than with the radiometric method. This may be due to the fact that formate oxidation can be blocked by a simple action on formate dehydrogenase, whereas the blockade of protein synthesis and genetic function involves drug action on more complex steps.

The generation times found with the radiometric method were slightly lower than values reported in the literature [2]. This could be due in part to a possible elongation of the organisms, a phenomenon which accounts for increased $^{14}CO_2$ output not necessarily followed by cell division. In any circumstance, however, the ^{14}C-substrates used to monitor **M. tuberculosis** metabolism seem to be adequate on the basis of the generation times obtained.

RESUMO

Estudos radiométricos com o Mycobacterium tuberculosis.

A atividade metabólica do **M. tuberculosis** sob diversas condições experimentais foi estu-

dada utilizando um sistema radiométrico automático, capaz de quantificar o $^{14}CO_2$ produzido pela oxidação de substâncias marcadas com carbono-14. As experiências realizadas incluíram: a) vias metabólicas; b) determinação dos tempos de detecção para inoculações de diversas magnitudes; c) efeito da filtração sobre a reprodutibilidade dos resultados; d) influência de meio hostil; e) determinação das concentrações inibitórias mínimas para hidrazida, estreptomicina, etambutol e rifampicina; f) tempo de duplicação para o **M. tuberculosis** e **M. bovis**. Estes microorganismos metabolizaram até $^{14}CO_2$ o ^{14}C-formato, (U-^{14}C) acetato, (U-^{14}C) glicerol, (1-^{14}C) ácido palmítico, (1-^{14}C) ácido láurico, (U-^{14}C) L-ácido málico, (U-^{14}C) D-glicose e (1-^{14}C) D-glicose, mas não (1-^{14}C) L-glicose, (U-^{14}C) glicina ou (U-^{14}C) piruvato. Usando ^{14}C-formato, (1-^{14}C) ácido palmítico ou (1-^{14}C) ácido láurico foi possível detectar 10 bacilos/frasco em 24-48 horas e até 10 bacilos/frasco em 16-20 dias. Resultados reprodutíveis foram obtidos sem filtrar a suspensão de bactérias, desde que cultivadas em meio 7H9 líquido com 0,05% de polissorbato 80 e homogenizadas antes da experiência. Drogas que bloqueiam a síntese protéica apresentaram concentração inibitória mínima menor com o método radiométrico do que com o convencional. O tempo médio de duplicação para o **M. bovis** e várias cepas de **M. tuberculosis** com diversas substâncias marcadas foi 9 ± 1 horas.

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REFERENCES

1. BAILEY, W. R. & SCOTT, E. G. — **Diagnostic microbiology**. 2nd. ed. St. Louis, C.V. Mosby, 1970c. p. 368.

2. BUCHANAN, R. E. & GIBBONS, N. E., ed. — **Bergey's manual of determinative bacteriology**. 8th. ed. Baltimore, Williams and Wilkins, 1974. p. 683.

3. BUDDEMEYER, E. U.; HUTCHINSON, R. & COOPER, M. — Automatic quantitative radiometric assay of bacterial metabolism. **Clin. Chem.**, 22: 1459-1464, 1976.

4. CAMARGO, E. E.; LARSON, S. M.; TEPPER, B. S. & WAGNER JR., H. N. — Radiometric measurement of metabolic activity of **Mycobacterium lepraemurium**. **Appl. Microbiol.**, 28: 452-455, 1974.

5. CAMARGO, E. E.; LARSON, S. M.; TEPPER, B. S. & WAGNER JR., H. N. — A radiometric method for predicting effectiveness of chemotherapeutic agents in murine leprosy. **Int. J. Leprosy**, 43: 234-238, 1975.

6. CAMARGO, E. E.; CHARACHE, P.; LARSON, S. M.; KERTCHER, J.; HWANGBO, C. C. & WAGNER JR., H. N. — Radiometric testing of susceptibility of **M. tuberculosis** to drugs. In: **Interscience Conference on Amtimicrobial Agents and Chemotherapy**, 15., Washington, D.C., September 1975.

7. CAMARGO, E. E.; LARSON, S. M.; TEPPER, B. S. & WAGNER JR., H. N. — Radiometric studies of **Mycobacterium lepraemurium**. **Int. J. Leprosy**, 44: 294-300, 1976.

8. CAMARGO, E. E. & LARSON, S. M. — Radiomicrobiology. In: ROCHA, A. F. G. & HARBERT, J. C., ed. — **Textbook of nuclear medicine**: basic science. Philadelphia, Lea & Febiger, 1984. p. 339.

9. CUMMINGS, D. M.;; RISTROPH, D.; CAMARGO, E. E.; LARSON, S. M. & WAGNER JR., H. N. — Radiometric detection of the metabolic activity of **Mycobacterium tuberculosis**. **J. nucl. Med.**, 16: 1189-1191, 1975.

10. DeBLANC JR., H. J.; CHARACHE, P. & WAGNER JR., H. N. — Automated radiometric measurement of antibiotic effect on bacterial growth. **Antimicrob. Agents Chemother.**, 2: 360-336, 1972.

11. DEHYLE, R. L. & BARTON, L. L. — Nicotinamide adenine dinucleotide-independent formate dehydrogenase in **Mycobacterium phlei**. **Canad. J. Microbiol.**, 23: 125-130, 1977.

12. DeLAND, F. H. & WAGNER JR., H. N. — Automated radiometric detection of bacterial growth in blood cultures. **J. Lab. clin. Med.**, 75: 529-534, 1970.

13. DeLAND, F. H. — Bacteriologic cultures and sensitivities. In: ROTHFELD, B., ed. — **Nuclear medicine in vitro**. Philadelphia, J.B. Lippincot, 1974c. p. 384.

14. KERTCHER, J. A.; CHEN, M. F.; CHARACHE, P.; HWANGBO, C. C.; CAMARGO, E. E.; McINTYRE, P. & WAGNER JR., H. N. — Rapid radiometric susceptibility testing of **Mycobacterium tuberculosis**. **Amer. Rev. resp. Dis.**, 117: 631-637, 1978.

15. MIDDLEBROOK, G. & COHN, M. L. — Bacteriology of tuberculosis: laboratory methods. **Amer. J. publ. Hlth.**, 48: 844, 1958.

16. MIDDLEBROOK, G. — Personal communication.

17. MIDDLEBROOK, G.; REGGIARDO, Z. & TIGERTT, W. — Automatable radiometric detection of growth of **Mycobacterium tuberculosis** in selective media. **Amer. Rev. resp. Dis.**, 115: 1066-1069, 1977.

18. MORGAN, M. A.; HORSTMEIER, C. D.; DeYOUNG, D. R. & ROBERTS, G. D. — Comparison of a radiometric method (BACTEC) and conventional culture me-

dia for recovery of mycobacteria from smear-negative specimens. **J. clin. Miicrobiol.**, 18: 384-388, 1983.

19. SIDDIQI, S. H.; HWANGBO, C. C.; SILCOX, V.; GOOD, R. C.; SNIDER, D. E. & MIDDLEBROOK, G. — Rapid radiometric methods to detected and differentiate **Mycobacterium tuberculosis/M. bovis** from other mycobacterial species. **Amer. Rev. resp. Dis.**, 130: 634-640, 1984.

20. TEPPER, B. S. & VARMA, K. G. — Metabolic activity of purified suspensions of **Mycobacterium lepraemurium. J. gen. Microbiol.**, 73: 143-152, 1972.

21. WEISS, E. — Adenosine triphosphate and other requirements for the utilization of glucose by agents of the psittacosis-tracoma group. **J. Bact.**, 90: 243-253, 1965.

22. YANGCO, B. G.; EIKMAN, E. A.; SOLOMON, D. A.; DERESINSKI, S. C. & MADDEN, J. A. — Rapid radiometric method for determining drug susceptibility of **Mycobacterium avium-intracellulare. Antimicrob. Agents Chemother.**, 19: 534-539, 1981.

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