*Rev. Inst. Med. trop. São Paulo*
**29**(1):9-17, *janeiro-fevereiro*, 1987

# RADIOMETRIC STUDIES ON THE OXIDATION OF (1-$^{14}$C) FATTY ACIDS BY DRUG-SUSCEPTIBLE AND DRUG-RESISTANT MYCOBACTERIA

Edwaldo E. CAMARGO, M.D., Theresa M. KOPAJTIC, Glinda K. HOPKINS, Nancy P. CANNON & Henry N. WAGNER JR., M.D.

## ABSTRACT

A radiometric assay system has been used to study oxidation patterns of (1-$^{14}$C) fatty acids by drug-susceptible and drug-resistant organisms of the genus **Mycobacterium.**

Two strains of **M. tuberculosis** susceptible to all drugs, $H_{37}Rv$ and Erdman, were used. Drug-resistant organisms included in this investigation were **M. tuberculosis** $H_{37}Rv$ resistant to 5 ug/ml isoniazid, **M. bovis, M. avium, M. intracellulare, M. kansasii** and **M. chelonei.** The organisms were inoculated in sterile reaction vials containing liquid 7H9 medium, 10% ADC enrichment and 1.0 uCi of one of the (1-$^{14}$C) fatty acids (butyric, hexanoic, octanoic, decanoic, lauric, myristic, palmitic, stearic, oleic, linoleic, linolenic). Vials were incubated at 37°C and the $^{14}CO_2$ envolved was measured daily for 3 days with a Bactec R-301 instrument.

Although each individual organism displayed a different pattern of fatty oxidation, these patterns were not distinctive enough for identification of the organism. No combination of fatty acids nor preferential oxidation of long chain or of short chain fatty acids were able to separate susceptible from resistant organisms. Further investigation with a larger number of drug susceptible mycobacteria including assimilation studies and oxidation of other substrates may be required to achieve a distinction between drug-susceptible and drug-resistant mycobacteria.

**KEY WORDS:** Mycobacteria; $^{14}$C-Fatty Acids; Oxidation patterns; Radiometric system; Drug-resistance.

## INTRODUCTION

The idea of using carbon-14 labeled fatty acids as substrates to study mycobaterial metabolism resulted from an incidental finding in one of the experiments with **M. lepraemurium** [5]. It was observed that polysorbate 80 markedly enhanced the oxidation of (U-$^{14}$C) acetate by this organism. It was assumed that oleic acid, which is part of the chemical structure of polysorbate 80, was responsible for increased oxidation rates of (U-$^{14}$C) acetate. An investigation of the oxidation rate of (1-$^{14}$C) oleic acid revealed a $^{14}CO_2$ production comparable to that of (U-$^{14}$C) acetate and led to a study of the entire series of (1-$^{14}$C) fatty acids.

Because some radiometric similarities had already been found with **M. lepraemurium** and **M. tuberculosis** [4], the study of the (1$^{14}$C) fatty

From the Division of Nuclear Medicine, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205, U.S.A.
Supported in part by USPHS Grant GM 10548.
**Address for correspondence:** Edwaldo E. Camargo, M.D., Centro de Medicina Nuclear da Universidade de São Paulo. Caixa Postal 22022. CEP 01499 São Paulo, SP., Brasil.

acid series was extended to include the latter. Preliminary experiments showed differences in the oxidation rates of fatty acids between these organisms and led to the hypothesis that oxidation patterns of fatty acids might provide a basis for differentiation of mycobacteria. For example, a comparison of oxidation patterns of **M. lepraemurium, M. bovis, M. tuberculosis** ($H_{37}Rv$ and Erdman) showed significant differences [6,7]. Also, preliminary results with isoniazid-resistant and isoniazid-susceptible strains of **M. tuberculosis** suggested that oxidation patterns of fatty acids and amino acids might help to identify susceptible and resistant organisms [8].

The present study describes a series of experiments in which oxidation of (1-$^{14}$C) fatty acids was used in an attempt to differentiate susceptible and resistant strains of mycobacteria.

## MATERIALS AND METHODS

**Preparation of Bacilli:** The organisms obtained from Trudeau Mycobacterial Cultura Collection (Saranac Lake, N.Y.) included **M. tuberculosis** $H_{37}Rv$ (TMC 102) fully susceptible; **M. tuberculosis** $H_{37}Rv$ (TMC 303) resistant to 5 ug/ml isoniazid (INH); **M. tuberculosis** Erdman (TMC 107) fully susceptible; **M. bovis** (TMC 602) resistant to 5 ug/ml INH; **M. avium** (TMC 706) resistant to 5 ug/ml INH, 10 ug/ml INH, 10 ug/ml para-amino salicylic acid (PAS), 2 ug/ml streptomycin (SM), 5 ug/ml viomycin (VM), 5 ug/ml kanamycin (KM), 30 ug/ml cycloserine (CS), 5 ug/ml ethionamide (ETA), 12 ug/ml ethambutol (EMB), 5 ug/ml rifampin (RIF), 50 ug/ml pyrazinamide (PZA), susceptible to all others; **M. intracellulare** (TMC 1403) resistant to 5 ug/ml INH, 10 ug/ml PAS, 2 ug/ml SM, 5 ug/ml KM, 5 ug/ml VM, 30 ug/ml CS, 6 ug/ml EMB, 0.2 ug/ml RIF, 50 ug/ml PZA, susceptible to all others; **M. kansasii** (TMC 1201) resistant to 1 ug/ml INH, 2 ug/ml SM, 6 ug/ml EMB, 0.2 ug/ml RIF, 25 ug/ml PZA, susceptible to all others; **M. chelonei** (TMC 1542) resistant to INH, SM, PAS, VM, CS, ETA, RIF, PZA, susceptible to 50 ug/ml KM. All organisms were received in 3 ml sterile serum vials containing 2 ml of bacterial suspension in liquid 7H9 medium (Baltimore Biological Laboratories, BBL). These suspensions were immediately transferred to sterile 250 ml erlenmeyer flasks with gauze plugs, containing 50 ml of liquid 7H9 medium with 10% albumin-dextrose-catalase (ADC, BBL) enrichment and 0.05% polysobate 80. The organisms were incubated at 37°C for 6 to 11 days and then homogenized with a Sorval Omnimixer (8.5 speed scale) twice for 30 seconds. The number of bacteria was estimated using MacFarland barium sulfate standards [2]. The final suspension was further diluted with sterile 7H9 medium to yield from 1 x $10^8$ to 6 x $10^8$ bacteria per ml. Part of this suspension was again diluted 1:10 with 7H9 medium, divided into 2 ml aliquots dispensed in sterile plastic tubes and stored at -70°C for future experiments.

**Experimental Media:** Liquid 7H9 medium with 10% ADC enrichment and no polysorbate was used with all the organisms tested.

**Reaction System:** The reaction system for detection of $^{14}CO_2$ consisted of 0.8 ml medium in a 5.0 ml multidose sterile glass vial with 0.1 ml of bacterial suspension and 0.1 ml (1.0 uCi) of $^{14}$C-substrate. The following (1-$^{14}$C) fatty acids (Amersham Corporation) were used with all organisms: butyric, hexanoic, octanoic, decanoic, lauric, myristic, palmitic, stearic, oleic, linoleic, and linolenic. In preliminary experiments [9], (1-$^{14}$C) fatty acids were dissolved in ethyl alcohol or methyl alcohol (lauric). A comparison of $^{14}CO_2$ output from alcohol dissolved and bovine serum albumin (BSA) — catalase complex dissolved (1-$^{14}$C) fatty acid demonstrated a better oxidation rate when using the latter (Fig. 1). In the present study, all uCi of (1-$^{14}$C) fatty acid were dissolved in 1.0 fatty acids were prepared as follows: a) 250 uCi of (1-$^{14}$C) fatty acid were dissolved in 1.0 ml of 0.01 N KOH; b) 1.0 ml of a 30% solution of BSA in Tyrode's solution was added; c) 0.05 ml of 0.1 N HCl were added and pH checked between 6.5 and 7.0; d) the solution was filtered through a 0.22 u pore size membrane filter (Millipore Corporation); e) 0.25 ml of sterile catalase (50,000 — 59,000 units/ml) were added; f) sterile water was added to bring the final solution to desired volume. All specific activities were lowered to 25 mCi/mM by addition of the corresponding non-radioactive fatty acid.

As indicators of metabolic activity of the organisms, $^{14}$C-formate (25 mCi/mM) and

(U-$^{14}$C) acetate (25 mCi/mM) were used [16]. All vials were prepared at least in triplicate. Control vials were prepared in the same way, but with autocloved bacteria added. Each experiment was repeated at least twice.

**Radiometric Measurement:** All vials were incubated at 37°C. An ion chamber device (Bactec R-301, Johnston Laboratories, Cockeysville, Md.) was used to measure bacterial metabolism. The vials were sampled daily for 3 days. Results were obtained as "index units" (100 units = 25 nanocuries of $^{14}$C activity). Mean and standared devidation of the cumulative $^{14}CO_2$ production for each substrate over the entire experimental period were calculated. Results were expressed as percent lauric acid oxidation.

**Sterility Testing:** Sterility tests were performed on positive samples and consisted of subculture on chocolate-agar plates and radiometric sterility testing with (U-$^{14}$C) glucose [10,11].

## RESULTS

Figure 1 shows a comparison of $^{14}CO_2$ output from alcohol dissolved and BSA-catalase complex dissolved (1-$^{14}$C) fatty acids with **M. tuberculosis** $H_{37}Rv$ fully susceptible (TMC 102) Higher $^{14}CO_2$ production rates were obtained when the fatty acids were dissolved in BSA-catalase complex. Similar results were found with **M. avium** and **M. tuberculosis** $H_{37}Rv$ resistant to 5 ug/ml INH (TMC 303).

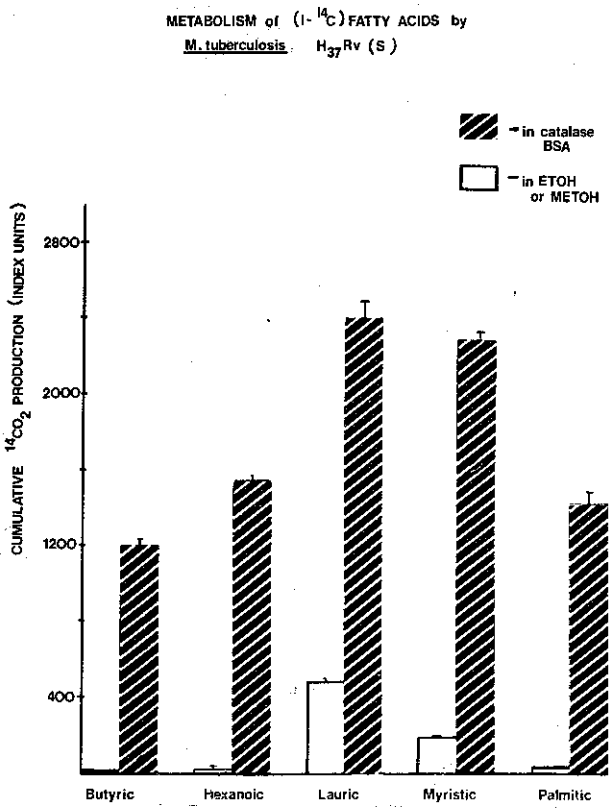


Fig. 1 — Influence of BSA-catalase complex on the oxidation rates of (1-$^{14}$C) fatty acids by **M. tuberculosis** $H_{37}Rv$ fully susceptible to drugs (TMC 102) in 7H9 medium.

When exposed to the entire (1-$^{14}$C) fatty acids series, **M. tuberculosis** $H_{37}Rv$ fully susceptible (TMC 102) showed highest oxidation rates with octanoic, decanoic, lauric, and myristic acids. Low oxidation rates were found with butyric, hexanoic, linoleic and oleic acids (Fig. 2, Table I).

**M. tuberculosis** Erdman showed preferential oxidation with hexanoic, lauric, palmitic and linoleic acids. Intermediate oxidation rates occurred with decanoic, myristic, stearic and oleic acids. Lower oxidation rates were found with butyric, octanoic and linolenic acids. (Fig. 2, Table I).

**M. tuberculosis** $H_{37}Rv$ resistant to 5 ug/ml INH (TMC 303) showed two distinct sets of consecutive fatty acids with high oxidation rates: octanoic, decanoic, lauric, and myristic acids; stearic, oleic and linoleic acids. Intermediate oxidation rates occurred with butyric, hexanoic, palmitic and linolenic acids (Fig. 2, Table I).

Except for butyric, hexanoic and linolenic acids, high oxidation rates were found for all other fatty acids with **M. bovis** (Fig. 2, Table I), especially decanoic and octanoic.

**M. kansasii** showed intermediate oxidation rates with palmitic, oleic, linoleic and linolenic acids. High oxidation rates were found with all other fatty acids, especially myristic (Fig. 3, Table I). Hexanoic acid was not tested with this organism.

I contrast, **M. chelonei** showed low oxidation rates with hexanoic and myristic acids, intermediate rate with linolenic and high oxidaton rates with the remaining fatty acids (Fig. 3, Table I).

CAMARGO, E. E.; KOPAJTIC, T. M.; HOPKINS, G. K.; CANNON, N. P. & WAGNER JR., H. N. — Radiometric studies on the oxidation of (1-$^{14}$C) fatty acids by drug-susceptible and drug-resistant mycobacteria. **Rev. Inst. Med. trop. São Paulo,** 29(1):9-17, 1987.

T A B L E I

Oxidation atterns of (1-$^{14}$C) fatty acids by mycobacteria *

| | M. tuberculosis ($H_{37}$Rv-S) | M. tuberculosis (Erdman) | M. tuberculosis ($H_{37}$Rv-R) | M. bovis | M. kansasii | M. chelonei | M. intracellulare | M. avium |
|---|---|---|---|---|---|---|---|---|
| Butyric | 669 ± 173** | 796 ± 133 | 879 ± 57 | 228 ± 41 | 1477 ± 141 | 1693 ± 158 | 1529 ± 202 | 1243 ± 193 |
| Hexanoic | 626 ± 151 | 2547 ± 186 | 993 ± 191 | 249 ± 21 | NT | 609 ± 90 | NT | 1243 ± 123 |
| Octanoic | 1941 ± 237 | 1008 ± 265 | 1815 ± 96 | 1265 ± 83 | 1398 ± 141 | 1918 ± 248 | 2315 ± 202 | 1803 ± 315 |
| Decanoic | 2330 ± 216 | 1592 ± 212 | 2139 ± 76 | 1524 ± 394 | 1178 ± 188 | 1580 ± 203 | 1619 ± 180 | 1786 ± 350 |
| Lauric | 2157 ± 216 | 2653 ± 133 | 1910 ± 134 | 1037 ± 73 | 1571 ± 204 | 2257 ± 406 | 2248 ± 135 | 1751 ± 298 |
| Myristic | 2049 ± 151 | 1645 ± 239 | 1986 ± 267 | 1193 ± 93 | 1744 ± 157 | 813 ± 90 | 787 ± 90 | 1821 ± 158 |
| Palmitic | 1057 ± 129 | 2334 ± 186 | 1261 ± 134 | 1213 ± 145 | 1021 ± 79 | 2144 ± 354 | 2360 ± 247 | 2049 ± 368 |
| Stearic | 1553 ± 86 | 1565 ± 159 | 1776 ± 134 | 1234 ± 218 | 1650 ± 189 | 1625 ± 181 | 1776 ± 180 | 1576 ± 210 |
| Oleic | 798 ± 86 | 1751 ± 106 | 1967 ± 210 | 913 ± 135 | 880 ± 204 | 1715 ± 226 | 2270 ± 202 | 2294 ± 280 |
| Linoleic | 669 ± 86 | 2945 ± 239 | 2101 ± 191 | 1276 ± 73 | 911 ± 94 | 2392 ± 248 | 2765 ± 562 | 1751 ± 88 |
| Linolenic | 1100 ± 43 | 1141 ± 80 | 1070 ± 153 | 570 ± 104 | 927 ± 283 | 1151 ± 33 | 1529 ± 180 | 1138 ± 158 |

* Cumulative $^{14}CO_2$ production over 3 days as metabolic index units

** Mean ± standard deviation

NT = not tested

S = Susceptible to all drugs (TMC 102)

R = Resistant to 5 mg/ml isoniazid (TMC 303)

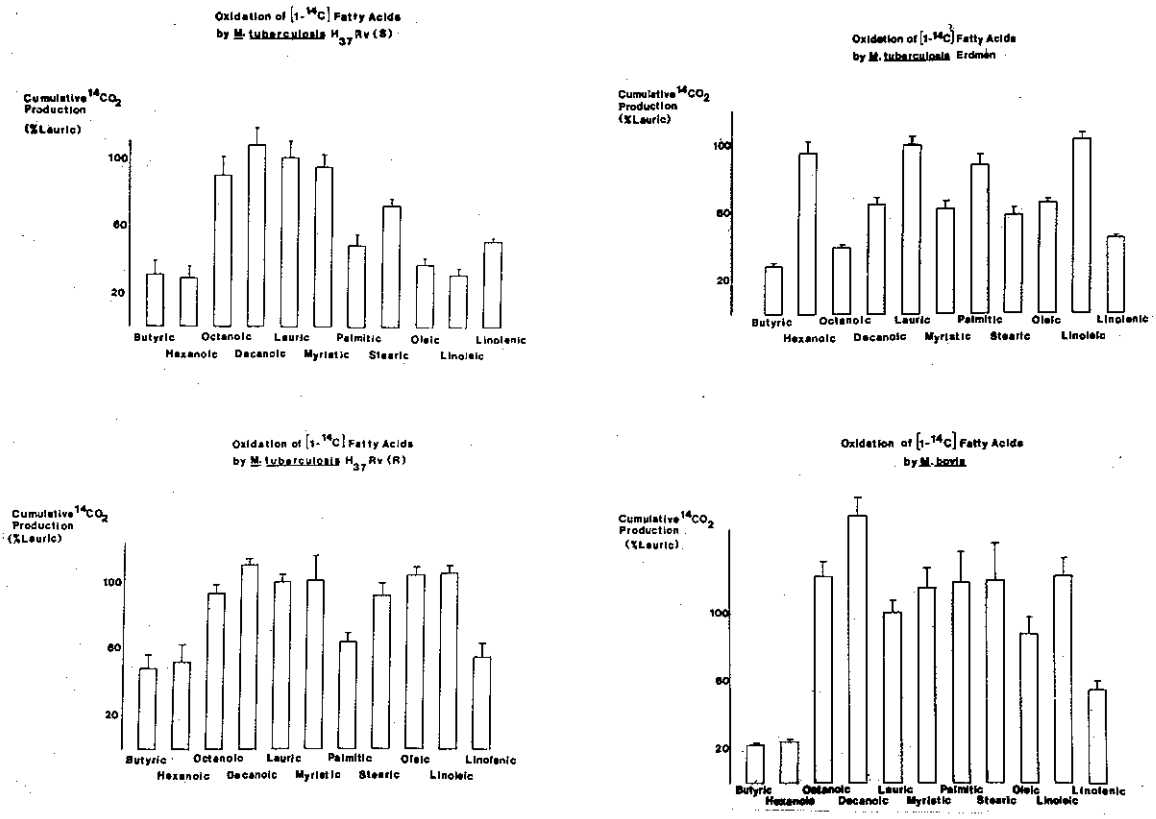


Fig. 2 — Pattern of (1-$^{14}$C) fatty acid oxidation by **M. tuberculosis** $H_{37}$Rv fully susceptible to drugs (upper left), **M. tuberculosis** Erdman ()upper right), **M. tuberculosis** $H_{37}$Rv resistant to 5 ug/ml isoniazid (lower left), and M, borts (lower right). All organisms were tested in liquid 7H9 medium with 10% ADC enrichment.

Linoleic acid was the best substrate with **M. intracellulare.** High oxidation rates were also found with octanoic, lauric, palmitic and oleic. Intermediate rates occurred with butyric, decanoic, stearic and linolenic. Myristic was poorly oxidized and hexanoic was not tested with this organism (Fig. 3, Table I).

**M. avium** oxidized the entire fatty acid series very avidly, particularly oleic and palmitic (Fig. 3, Table I).

In order to simplify the results presented in Table I and figures 2 and 3, an arbitrary threshold set at > 50% lauric acid oxidation

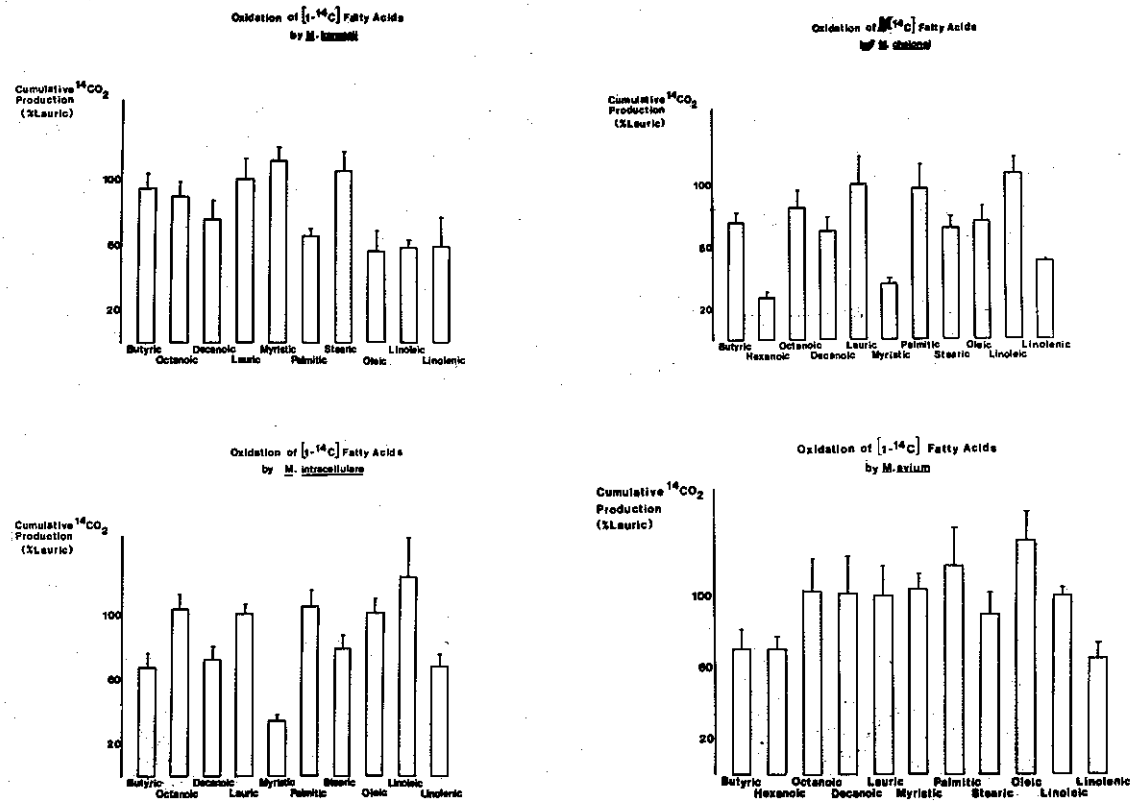


Fig. 3 — Pattern of (1-$^{14}$C) fatty acid oxidation by **M. kansasii** (upper left), **M. chelonei** (upper right), **M. intracellulare** (lower left), and **M. avium** (lower right). All organisms were tested in liquid 7H9 medium with 10% ADC enrichment.

was chosen to eliminate poorly oxidized substrates. Table II shows the results of this approach.

Five convenient substrates were chosen in Table II, with the purpose of differentiating the organisms used in this study. This simpler approach included butyric, myristic, oleic, linolenic and hexanoic (Table III). However, as can be observed in Table II, it was not possible to separate **M. tuberculosis** $H_{37}$Rv TMC 303 from **M. bovis.**

As expected, $^{14}CO_2$ production was not observed in the control vials and all sterility tests were negative.

## DISCUSSION

Fatty acid synthesizing enzyme activity has been found in extracts of several mycobacteria [13,18,21,24,25,27]. These studies have shown that a variety of long chain fatty acids are formed, from 12 to 26 carbons, by addition of two carbons to the acceptor fatty acid's carboxyl end. Acetate, malonate and NADH are essential for these reactions. There is also evidence that **M. tuberculosis** accumulates lipids during exponential growth [1], with a synthesis of mycolic acids that parallels the growth rate [3]. Some of the short and long chain fatty acids were preferably incorporated into trigly-

T A B L E II

(1-$^{14}$C) fatty acids with significant oxidation rates

| | M. tuberculosis ($H_{37}$Rv-S) | M. tuberculosis (Erdman) | M. tuberculosis ($H_{37}$Rv R) | M. bovis | M. kansasii | M. chelonei | M. intracellulare | M. avium |
|---|---|---|---|---|---|---|---|---|
| Butyric | 0 | 0 | 0 | 0 | + | + | + | + |
| Hexanoic | 0 | + | 0 | 0 | NT | 0 | NT | + |
| Octanoic | + | 0 | + | + | + | + | + | + |
| Decanoic | + | + | + | + | + | + | + | + |
| Lauric | + | + | + | + | + | + | + | + |
| Myristic | + | + | + | + | + | 0 | 0 | + |
| Palmitic | 0 | + | + | + | + | + | + | + |
| Stearic | + | + | + | + | + | + | + | + |
| Oleic | 0 | + | + | + | 0 | + | + | + |
| Linoleic | 0 | + | + | + | 0 | + | + | + |
| Linolenic | 0 | 0 | 0 | 0 | 0 | 0 | + | + |

+ = > 50% acid oxidation
0 = < 50% lauric oxidation
NT = not tested
S = Susceptible to all drugs (TMC 102)
R = Resistant to 5 ug/ml isoniazid (TMC 303)

T A B L E III

Oxidation patterns of selected (1-$^{14}$C) fatty acids by mycobacteria

| | Butyric | Myristic | Oleic | Linolenic | Hexanoic |
|---|---|---|---|---|---|
| **M. tuberculosis** ($H_{37}$Rv-S) | 0 | + | 0 | 0 | 0 |
| **M. tuberculosis** (Erdman) | 0 | + | + | 0 | + |
| **M. tuberculosis** ($H_{37}$Rv-R) | 0 | + | + | 0 | 0 |
| **M. bovis** | 0 | + | + | 0 | 0 |
| **M. kansasii** | + | + | 0 | 0 | NT |
| **M. chelonei** | + | 0 | + | 0 | 0 |
| **M. intracellulare** | + | 0 | + | + | NT |
| **M. avium** | + | + | + | + | + |

S = susceptible to all drugs (TMC 102)
R = resistant to 5 mg/ml isoniazid (TMC 303)

cerides [19], or into phospholipids [23] and retained in the cell whereas some of them were transformed into by-products and excreted into the medium [6,9].

The excretion of by-products into the medium may represent a mechanism of elimination of toxic substances. An example of such a toxic effect was observed with oleic acid: in high concentration it inhibited the growth of tubercle bacilli, but in low concentration it enhanced growth of this organism, as did butyric and decanoic [12]. This inhibitory effect caused by fatty acids on **M. tuberculosis** ($H_{37}$Rv) and **M. bovis** first increased with the carbon chain length to a maximum with decanoic and then decreased[12]. On the other hand, KONDO and KANAI [17], also studied the bactericital effect of fatty acids and found that myristic acid acid had the strongest lethal effect on **M. tuberculosis** $H_{37}$Rv and on **M. bovis**: longer and shorter chain fatty acids were less toxic than myristic. Such toxic effects or a possible inhibitory effect on bacterial enzymes involved in lipogenesis [14] probably would not occur with the small amounts of fatty acids used in our experiments (approximately 4 x $10^{-8}$M per vial). Therefore, one could only speculate whether or not the enzyme systems of the organisms included in our experiments are sensitive enough to recognize potentially toxic substances and either transform them into non-toxic triglycerides or excrete them as by-products.

Long chain fatty acids have been shown to have a stimulatory effect on oxygen uptake by **M. tuberculosis.** A similar mechanism could be present in other mycobacteria and would explain the various oxidation rates observed in our experiments. It is also conceivable that the magnitude of the stimulatory effect varies from one organism to another for the same fatty acid. Therefore, the preferential oxidation of one or more substrates over the others by a particular mycobacteria could be due to the toxic nature of the substrate, to the stimulatory effect on oxygen uptake, to a combination of both or because the substrate, for some reason, is particularly suitable for the energy needs of the organism.

Assimilation studies with **M. lepraemurium** and **M. tuberculosis** $H_{37}$Rv fully susceptible to drugs [6,7] allowed us to identify fatty acids used primarily as energy sources and those used primarily as carbon sources. We also found that adsorption of the fatty acids of higher molecular weight such as lauric, myristic, palmitic and stearic could have been a determinant of the assimilation process. With **M. tuberculosis** most of the substrates were used as both carbon sources and energy sources, whereas lauric and formate were used chiefly to provide energy. With **M. lepraemurium,** lauric was the best energy source and carbon-2, but not carbon-1 of acetate the best carbon source. Because assimilation studies with radiochromato-

graphic analysis were not performed in the present investigation, identification of the role each individual fatty acid was playing in each organism was not possible.

Studies of **M. avium** have shown that palmitic acid is necessary for maximal cell division rate and that cell division occurs when palmitic acid uptake ceases [19,20]. When **M. avium** was pregrown to filamentous stage in the presence of palmitic acid and then transferred to a fresh medium lacking fatty acid, the cells divided at a very slow rate and did not fragment [20]. This means that the elongated cells were not "committed" to fragmentation in the absence of palmitic acid. These findings suggest the presence of similar "commitments" to fragmentation with a particular fatty acid in other mycobacteria. An investigation of **M. avium** metabolism of the (1-$^{14}$C) fatty acid series would be revealing, particularly if one found palmitic acid as the preferred substrate for this organism. This might indicate that preferred $^{14}$C-substrates detected radiometrically are eventually the fatty acids to which the respective organisms are "committed" for cell division. Results of our experiments with **M. avium** showed that oleic and palmitic acids were the best substrates with this organism. Assessment of the role these fatty acids are playing in **M. avium's** metabolism could have been revealing if for example, palmitic acid was found to be more important as carbon source than energy source. Unfortunately, assimilation estudies and radiochromatographic analysis with this organism were not done in our experiments.

The enzyme systems of the various organisms used in this study did not oxidate the entire (1-$^{14}$C) fatty acid series at the same rates. Moreover, the different oxidation rates for the various fatty acids also varied from one organism to another. Therefore, to some extent, differential oxidation patterns could be recognized. Although each individual organisms displayed a different pattern of fatty acid oxidation, these patterns were not distinctive enough for identification of the organism. For example, a combination of poor oxidation of butyric and hexanoic which could be distinctive of **M. tuberculosis** $H_{37}Rv$ fully susceptible to drugs (TMC 102), was also found with **M. bovis**. Very poor oxidation of myristic was an infrequent finding, but could not be used to differentiate **M. intracellulare** because **M. chelonei** showed the same pattern. In our preliminary studies, a comparison of **M. tuberculosis** $H_{37}Rv$ fully susceptible with **M. tuberculosis** $H_{37}Rv$ resistant to 5 ug/ml INH and **M. avium**, suggested that the susceptible strain preferred short chain fatty acids, whereas the drug-resistant organisms oxidized long chain fatty acids also. However, as other drug-resistant mycobacteria were added to our study, a trend similar to that of **M. tuberculosis** $H_{37}Rv$ fully susceptible was also noted with **M. kansasii**. These findings suggested to us that the use of a threshold was needed to attempt differentiation of these organisms. Arbitrarily, only fatty acid oxidation rates $> 50\%$ lauric acid oxidation were considered significant. When this threshold was used, most of the organisms in our study could be identified; however, it also became apparent that a distinction between **M. tuberculosis** $H_{37}Rv$ (TMC 303) and **M. bovis** was impossible, using fatty acids.

Pathogenicity and virulence of mycobacteria seem to be related to fatty acid content. The presence of an attenuation lipid in attenuated strains of mycobacteria, but absent in virulent strains has been described by GOREN at al [15]. NANDEKKAR [22] found that most pathogenic mycobacteria have a higher content of saturated fatty acids when compared to the unsaturated ones. It has been demonstrated that INH uptake by tubercle bacilli, an enzyme-dependent phenomenon [26], is followed by a fall in the incorporation of (1-$^{14}$C) acetate into fatty acids [13]. This supports the concept that there is some relationship between drug action and fatty acid metabolism. It suggested to us that INH-resistant and INH-susceptible organisms of the same strain of **M. tuberculosis** might show different patterns of fatty acid oxidation. In this case, a comparison of oxidation patterns of selected fatty acids by these organisms would bring new information on metabolic pathways involved with the mechanism of susceptibility and resistance to INH. **M. tuberculosis** $H_{37}Rv$ (TMC 102) and **M. tuberculosis** $H_{37}Rv$ (TMC 303) were chosen for our investigation because the only difference between these organisms is INH resistance by the latter. Our results showed higher oxidation rates of long chain fatty acids, especially oleic and

linoleic with the resistant organism. This suggests that resistance to isoniazid may be related to the utilization of long chain fatty acids.

Our results have demonstrated that too many substrates may be required to discriminate a large number of mycobacteria. Preferential oxidation of long chain fatty acids or preferential oxidation of short chain fatty acids were not found to be distinctive enough to separate susceptible from resistant organisms. Further investigation with a larger number of susceptible mycobacteria including assimilation studies and oxidation of other substrates may be required in order to achieve a distinction between drug-susceptible and drug-resistant mycobacteria.

## RESUMO

### Esudos radiométricos sobre a oxidação de (1-$^{14}$C) ácidos graxos por micobactérias sensíveis e resistentes a drogas.

Um sistema radiométrico foi utilizado para estudar os padrões de oxidação dos (1-$^{14}$C) ácidos graxos por microorganismos do gênero **Mycobacterium** sensíveis e resistentes a drogas. Foram usadas duas cepas do **M. tuberculosis** sensíveis a todas as drogas, $H_{37}Rv$ e Erdman. As micobactérias resistentes foram **M. tuberculosis** $H_{37}Rv$ resistente a 5 ug/ml de hidrazida, **M. bovis, M. avium, M. intracellulare, M. kansasii** e **M. chelonei.** As micobactérias foram inoculadas em frascos estéreis contendo o meio líquido 7H9 com 10% do complexo albumina-dextrose-catalase e 1,0 uCi de um dos (1-$^{14}$C) ácidos graxos (butírico, hexanoico, octanoico, decanóico, láurico, mirístico, plamítico, esteárico, olêico, linolêico, linolênico). Os frascos foram incubados a 37°C e o $^{14}CO_2$ produzido pelas micobactérias foi medido durante 3 dias, com uma máquina Bactec-R-301.

Embora cada micobactéria apresentasse um padrão distinto de oxidação de ácido graxo, estes padrões não foram suficientemente diferentes para identificá-la. Nenhuma combinação de ácidos graxos nem a oxidação preferencial de ácidos graxos de cadeias longas ou curtas foi capaz de separar as micobactérias resistentes das sensíveis. Outras experiências com um maior número de micobactérias sensíveis, incluindo estudo da assimilação de substâncias marcadas, são necessárias para se tentar a diferenciação entre as micobactérias sensíveis e as resistentes a drogas.

## REFERENCES

1. ANTONINE, A. D. & TEPPER, B. S. — Enviromental control of glycogen and lipid content of **Mycobacterium tuberculosis. J. Bact., 100:** 538-539, 1969.

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

3. BENNET, P. & ASSELINEAU, J. — Influence de l'age sur la teneur en acides gras a chaine ramifie' du bacile tuberculeux. **Ann. Inst. Pasteur, 118:** 324-329, 1970.

4. CAMARGO, E. E.; LARSON, S. M.; TEPPER, B. S.; CHARACHE, P. & WAGNER JR., H. N. — Automated radiometric detection of **M. tuberculosis** and **M. lepraemurium. J. nucl. Med., 15:** 48, 1974.

5. 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.

6. CAMARGO, E. E.; KERTCHER, J. A.; LARSON, S. M.; TEPPER, B. S.; WAGNER JR., H. N. — Radiometric measurement of differential metabolism of fatty acids by **Mycobacterium lepraemurium. Int. J. Leprosy, 47:** 126-132, 1979.

7. CAMARGO, E. E.; KERTCHER, J. A. & LARSON, S. M. — Radiorespirometry in identification of mycobacteria. In: HORST, W.; WAGNER JR., H. N. & BUCHANAN, J. W., ed. — **Frontiers in Nuclear Medicine.** Berlin, Springer-Verlag, 1980. p. 286-298.

8. CAMARGO, E. E.; MILKOWSKI, T. A. & WAGNER JR., H. N. — Relationship between amino acid metabolism and drug resistance of **M. tuberculosis. J. nucl. Med., 21:** 59, 1980.

9. CAMARGO, E. E.; KERTCHER, J. A.; LARSON, S. M.; TEPPER, B. S. & WAGNER JR., H. N. — Radiometric measurement of differential metabolism of fatty acid by mycobacteria. **Int. J. Leprosy, 50:** 200-204, 1982.

10. DeBLANC JR., H. J.; DeLAND, F. H. & WAGNER JR., H. N. — Automated radiometric detection of bacteria in 2,967 blood cultures. **Appl. Microbiol., 22:** 846-849, 1971.

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

12. DUBOS, R. J. — The effect of organic acids on mammalian tubercle bacilli. **J. exp. Med., 92:** 319-332, 1950.

13. EBINA, T.; MUNAKATA, K. & MUTOMIYA, M. — Effect de l'isoniazide sur l'incorporation de l'acetate

de sodium 1-$^{14}$C dans les acides gras de la mycobacterie. **C. R. Soc Biol. (Paris), 155:** 1190-1192, 1961.

14. FERDINANDUS, J. & CLARKE, B. — Selective inhibition of bacterial enzymes by free fatty acids. **J. Bact., 98:** 1109-1113, 1969.

15. GOREN, M. B.; BROKL, O. & SCHAEFER, W. B. — Lipids of putative relevance to virulance in **Mycobacterium tuberculosis:** phthiocerol dimycocerosate and the attenuation indicator lipid. **Infect. Immun., 9:** 150-158, 1974.

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

17. KONDO, E. & KANAI, K. — The relationship between the chemical structure of fatty acids and their mycobactericidal activity. **Jap. J. med. Sci. Biol., 30:** 171-178, 1977.

18. KUSAKA, T. — Fatty acid synthesizing enzyme activity of cultured **Mycobacterium lepraemurium. Int. J. Peprosy, 45:** 132-138, 1977.

19. McCARTHY, C. — Utilization of palmitic acid by **Mycobacterium avium. Infect. Immun., 4:** 199-204, 1971.

20. McCARTHY, C. — Effect of palmitic acid utilization on cell division in **Mycobacterium avium. Infect. Immun., 9:** 363-372, 1974.

21. MEDHI, I.; MURTHY, P. S. & VENKITASUBRAMANIAN, T. A. — Heat-stable stimulating factor for fatty acid synthetase from **Mycobacterium tuberculosis** $H_{37}$Rv. **Amer. Rev. resp. Dis., 110:** 201-202, 1974.

22. NANDEKKAR, A. K. N. — Fatty acid composition of mycobacteria: An index of pathogenecity. **Fed. Proc., 33:** 1405, 1974.

23. OKUYAMA, H.; KAMEYAMA, Y.; FUJIKAWA, M.; YAMADA, K. & IKEZAWA, H. — Mechanism of diacylglycerophosphate synthesis in mycobacteria. **J. biol. Chem., 252:** 6682-6686, 1977.

24. PIÉRARD, A. & GOLDMAN, D. S. — Enzyme system in mycobacteria. 14. Fatty acid synthesis in cell-free extracts of **Mycobacterium tuberculosis. Arch. Biochem. Biophys., 100:** 56-65, 1963.

25. WANG, L.; KUSAKA, T. & GOLDMAN, D. S. — Elongation of fatty acids in **Mycobacterium tuberculosis. J. Bact., 101:** 781-785, 1970.

26. WIMPENNY, J. W. T. — The uptake and fate of isoniazid in **Mycobacterium tuberculosis** var **bovis** BCG. **J. gen. Microbiol., 47:** 389-403, 1967.

27. WINDER, F. G.; BRENNAN, P. & RATLEDGE, C. — Synthesis of fatty acids by extracts of mycobacteria and the absence of inhibition by isoniazid. **Biochem. J., 93:** 635-640, 1964.

Recebido para publicação em 6/2/1986.