A Radiometric Method for Predicting Effectiveness of Chemotherapeutic Agents in Murine Leprosy


Drug susceptibility of *M. lepraemurium* has been extensively used to test drug effectiveness against *M. lepra* (2-9, 14-18). This model is used because of the biologic similarities of *M. lepraemurium* and *M. lepra*, the causative agents of murine and human leprosy respectively (18, 21). Although neither organism can be cultured in cell-free media, *M. lepraemurium* is easier to study in the laboratory because it can be grown more conveniently in larger numbers in murine hosts.

Most of the models to evaluate *M. lepraemurium* susceptibility to drugs involve inoculation into mice, and since the organisms grow slowly, at least three months of observation are required before drug effect can be determined (7).

In 1969, a sensitive radiometric method for the detection of bacterial metabolism was introduced by DeLand and Wagner (12, 13). The technic measures $^{14}$CO$_2$ produced by the bacterial metabolism of $^{14}$C-labeled substrates. Recently, it has been shown that pure suspensions of *M. lepraemurium* are metabolically active (21). Based on these observations, a radiometric method has been developed which permits rapid detection of the metabolism of *M. lepraemurium* by measuring the $^{14}$CO$_2$ produced by conversion of acetate- U-$^{14}$C or glycerol- U-$^{14}$C (1).

The current paper reports the application of this radiometric technic to the rapid evaluation of the susceptibility of *M. lepraemurium* to a series of drugs.

**MATERIALS AND METHODS**

**Preparation of the bacilli.** *M. lepraemurium* (Hawaiian strain) was harvested from infected livers of female CBA/J mice (Jackson Laboratories) which had been inoculated intraperitoneally and intravenously three to four months previously with $5 \times 10^8$ bacteria. The bacteria were separated from tissue components using the technic of Tepper and Varma (21). At the end of this procedure, the bacteria were suspended in sterile water at a final concentration of $1 \times 10^9$ bacteria/ml.

**Buffer system.** The simple K-36 buffer of Weiss (22) (0.1M KCl, 0.01M NaCl, 0.05M KH$_2$PO$_4$, pH 7.0) was used as a buffer system for the bacteria.

**Drugs.** The effect of the following drugs on the metabolism of *M. lepraemurium* was studied: bacitracin, cephaloridine, chloramphenicol, cycloserine, dactinomycin, dapsone (DDS), ethionamide, isoniazid (INH), kanamycin, methenamine mandelate (mandelamine), nitrofurantoin, oxacillin, polymyxin B, rifampin (rifampicin), streptomycin, sulfa- diamethoxine, and vancomycin (see Appendix). Except for dactinomycin (0.5 mg/vial), the dose for all of the drugs was 5 mg/vial.

In a subsequent experiment, a combination of nitrofurantoin and ethionamide (5 mg/vial, each) was inoculated into four flasks containing $1 \times 10^9$ bacteria, suspended in K-36 buffer and into three flasks containing the complex NC-5 medium (1); four extra controls, two for each suspending medium, with the drugs were also prepared. After 15 days of incubation, the contents of these vials were pooled and 0.1 ml was inoculated intraperitoneally into three CFW female mice. The same was done with the suspensions of the control vials.

**Reaction system.** In order to detect bacterial metabolism, 10 ml of K-36 buffer were placed in a 20 ml multitube vial, along with 5 &mu;Ci of acetate-U-$^{14}$C (New England Nuclear), 1 ml of the final suspension of bacteria ($1 \times 10^9$ bacteria/vial) and the drug. All flasks were prepared in duplicate.
Controls without drugs were prepared in the same manner. In order to evaluate background levels and substrate stability, additional controls were prepared by adding autoclaved bacteria to vials which contained K-36 buffer and acetate-U-14C.

Radiometric measurement. Vials were incubated at 30° C and the 14C02 produced by bacterial metabolism was measured radiometrically. The details of operation of the measurement device (Bactec R-301, Johnston Laboratories), have been published previously (1). Measurement of the 14CO2 produced in each vial was begun at 24 hours and continued until 15 days after inoculation. In order to evaluate the effect of a particular drug on the metabolism of M. lepraemurium, the sum of the 14CO2 produced in the presence of drug over the 15 day testing interval was compared to that produced in the control vials without drug. The data were expressed as percent control radioactivity or as "index unit" (where 100 = 0.025 μCi) representing the cumulative 14CO2 production (1).

Sterility testing. Sterility tests were performed on all samples and consisted of subculture on chocolate-agar and radiometric sterility testing with glucose-U-14C (10-13).

RESULTS

Several drugs inhibited the metabolism of M. lepraemurium as shown in Figure 1. The inhibitory effect of the drugs increased as follows: bacitracin, rifampin, cephaloridine, DDS, kanamycin, sulfadimethoxine, oxacillin, vancomycin, polymyxin B, cycloserine, mandelamine, chloramphenicol, INH, ethionamide and nitrofurantoin.

Dactinomycin and streptomycin seemed to stimulate the metabolism of M. lepraemurium. Ethionamide and nitrofurantoin had the most marked inhibitory effect on the metabolism of these bacteria (Fig. 1). INH and chloramphenicol also had a significant inhibitory action.

The time course curves for the most effective drugs, ethionamide and nitrofurantoin are shown in Figure 2. The combination of these drugs, tested in a separate experiment was effective both in the K-36 buffer (Fig. 3) and in the NC-5 medium. In the K-36 buffer, after 15 days the activity in the vials which contained the combination of drugs was less than 5% of that of the controls (Fig. 3); in the NC-5 medium, the activity was about 14% of that of the controls after 13 days (Fig. 4).

Four months later, the mice injected with drug-treated bacteria were free of lepromas; the control mice showed a small leproma in
the site of injection. However, no histologic assessment was done on the sites of injection either in the drug-treated or in the control mice.

As expected, $^{14}$CO$_2$ production was not observed in the vials inoculated with autoclaved bacteria. All sterility tests were negative.

**DISCUSSION**

The *in vivo* effect of a number of drugs on the growth of *M. lepraemurium* has been studied exhaustively in a mouse model by Chang (22), Hadler (14), Hadler and Mauri (15, 16) and by Mauri et al (17). The techniques employed in these studies required observation of the animals for a minimum of three months for interpretation of the effect of the drug. In contrast, the radiometric technique which is described in this paper determined the effect of 17 drugs on the metabolism of *M. lepraemurium* within 15 days. The data obtained were used to rank the drugs from maximum to minimum inhibition of the metabolism of these organisms (Fig. 1).

It is presumed that a positive radiometric test is important information because it would indicate that at least one of the metabolic reactions of *M. lepraemurium* would be blocked. However, drugs not affected by the radiometric method should be further tested by methods involving active multiplication of these organisms. Therefore, it seems that this method has potential at least as a simple test for rapidly screening a large number of drugs or combination of drugs which might be effective against murine leprosy.

**SUMMARY**

A simple radiometric method has been developed for evaluating the effect of drugs on the metabolism of *M. lepraemurium*. The method is based on the measurement of the $^{14}$CO$_2$ produced through bacterial metabolism of acetate-$U-^{14}$C. Seventeen drugs were tested: bacitracin, cephaloridine, chloramphenicol, cycloserine, dactinomycin, DDS, ethionamide, INH, kanamycin, methenamine mandelate, nitrofurantoin, oxacillin, polymyxin B, rifampicin, streptomycin, sulfadimethoxine and vancomycin. The drugs which caused most marked inhibition were chloramphenicol, INH, ethionamide and nitrofurantoin in order of increasing effectiveness.

The radiometric study which is completed in 15 days permits direct study of the drug effect on the metabolism of *M. lepraemurium* and a more rapid screening of antileprosy drugs than has previously been possible. Currently, these observations are being extended to studies of the structure-activity relationships of antileprosy drugs and the metabolism and drug susceptibility of *M. leprae* *in vitro*. 

**FIG. 4.** Susceptibility of *M. lepraemurium* to a combination of nitrofurantoin and ethionamide in NC-5 medium.
RESUMEN
Se ha desarrollado un método radiométrico simple para evaluar el efecto de las drogas en el metabolismo del M. lepraemurium. El método está basado en la medición del 14CO₂ producido a través del metabolismo bacteriano de acetato-U-14C. Se estudiaron 17 drogas: bacitracina, cefaloridina, cloramfenicol, cicloserina, dactomicina, mandelato de metenamina, nitrofurantoina, oxacilina, polymixina B, rifampicina, estreptomicina, sulfadimetoxina y vancomicina. Las drogas que causaron la inhibición más marcada fueron el cloramfenicol, INH, etionamida y nitrofurantoina, en orden de incremento de efectividad.

El estudio radiométrico, que se completa en 15 días, permite un estudio directo del efecto de la droga sobre el metabolismo del M. lepraemurium y un estudio selectivo de drogas contra la lepra, más rápido que lo que habría sido posible hasta ahora. Actualmente, estas observaciones se están extendiendo a estudios sobre las relaciones estructura-actividad de drogas contra la lepra, y el metabolismo y la susceptibilidad a las drogas del M. leprae in vitro.

RÉSUMÉ
On a mis au point une méthode radiométrique simple pour évaluer l'action des médicaments sur le métabolisme de M. lepraemurium. La méthode est basée sur la mesure du CO₂ produit lors du métabolisme de l'acétate-U-14C chez les bactéries. Dix-sept médicaments ont été étudiés, à savoir le bacitracine, la céphaloridine, le chloramphénicol, la cyclodéérine, la dacty­micine, la DDS, l'éthionamide, l'INH, la kanamycine, le mandélate de méthénamine, la nitrofurantoïne, l'oxacilline, la polymyxine B, la rifampicine, la streptomycine, la sulfadiméthoxine et la van­comycine. Les médicaments ayant entraîné l'inhibition la plus prononcée étaient le chloram­phénicol, l'INH, l'éthionamide et la nitrofurantoïne, ces médicaments étant énumérés dans l'ordre d'une efficacité croissante.

L'étude radiométrique, qui a été achevée en 15 jours, permet une étude directe de l'action d'un médicament sur le métabolisme de M. lepraemurium, de même qu’un triage plus rapide des médicaments anti-lepréux qu'il n'avait été jusqu'à présent possible. On procède actuellement à un élargissement de ces observations, en vue d'étudier les relations entre la structure et l'activité des médicaments anti-lepréux, de même que le métabolisme et la susceptibilité aux médicaments de M. leprae in vitro.

APPENDIX
Drug Suppliers
Bacitracin: The Upjohn Company, Kalamazoo, Michigan.
Cephaloridine (Loridine): Eli Lilly and Company, Indianapolis, Indiana.
Chloramphenicol: Mann Research Laboratories, New York, New York.
Cycloserine: Mann Research Laboratories, New York, New York.
Dactinomycin (Cosmegen): Merek, Sharp and Dohme, West Point, Virginia.
DDS: Parke, Davis and Co., Detroit, Michigan.
Methenamine mandelate (Mandelamine): Warner Chilcott Laboratories, Morris Plains, New Jersey.
Nitrofurantoin: Sigma Chemical Company, St. Louis, Missouri.
Oxacillin (Bactocill): Beecham-Massengill Pharmaceuticals, Bristol, Tennessee.
REFERENCES
5. CHANG, Y. T. Chemotherapy of murine leprosy. IV. The effects of amithiozone (TB1/698), p-aminosalicylic acid (PAS), B2B3 (a phenazine pigment), five antibiotics and three diphenylthiourea compounds on mouse.
7. CHANG, Y. T. Chemotherapy of murine leprosy. VI. The effects of isonicotinylhydrazone of 2-carboxymethoxy-3-methoxybenzaldehyde (compound 373) and isonicotinylhydrazone of 2-carboxymethoxybenzaldehyde (compound 377) on mouse leprosy. Int. J. Lepr. 25 (1957) 120-146.