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Viable *M. leprae* as a Research Reagent[1]

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The viability of bacterial preparations can have a marked influence on experimental results. Replication and growth of bacilli has always been considered the gold standard for bacterial viability. Unfortunately, *Mycobacterium leprae* replicate only in living tissues, and the level of growth achieved by organisms inoculated into mouse foot pads (MFP) has been the only means to quantify viability of *M. leprae.* Such procedures require a high level of technical expertise and can yield results only several months after the bacilli already have been used in experiments. As a consequence, very few laboratories quantify *M. leprae* viability.

In recent times, a number of *in vitro* biochemical assays have been employed as screening aids for anti-*M. leprae* drug development. These assays do not measure replication of *M. leprae in vitro* but index their ATP content or uptake of radiolabeled carbon sources in axenic media. Since maintenance of metabolic activity is a hallmark of viability, detecting changes in the metabolic status of bacteria incubated in the presence of candidate drugs suggests a loss in viability and presumptive efficacy for the drug.

Our laboratory already has shown that oxidation of ^{14}C-palmitate by *M. leprae* in axenic culture is useful for drug screening. The technique detects changes in the metabolic activity of *M. leprae* manifest by drugs both *in vivo* and *in vitro,* and the assay has good qualitative association with MFP results ([5]).

In this study, we examined the utility of palmitate oxidation for detecting quantitative differences in the viability of *M. leprae* in comparison to conventional mouse foot pad methods. We also used the technique to describe the effect that many common laboratory procedures have on *M. leprae* viability in order to help highlight appropriate handling practices for viable *M. leprae* and to increase our understanding of this resource as a research reagent.

MATERIALS AND METHODS

Leprosy bacilli

Nude mouse-derived bacilli. *Mycobacterium leprae* (isolate T-53) was maintained in serial passage in the foot pads of athymic BALB/c *nu/nu* nude mice (Harlan, Indianapolis, Indiana, U.S.A.). Mice were inoculated on the plantar surface of both hind feet and harvested when their foot pads were moderately enlarged (0.5 g–1 g), and contained numerous rapidly growing bacilli.

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We harvested the bacilli using a procedure described previously ([1, 4]). Generally, the foot pads were cleansed in Betadine® and the skin removed before the highly bacilliferous tissue was excised and manually homogenized (Wheaton Science Products, Millville, New Jersey, U.S.A.) in 10 ml of 7H12 medium. Excess tissue was removed by slow-speed centrifugation, and the bacillary suspension was enumerated by direct count before aliquoting for further study according to a procedure described earlier ([15, 19]).

Armadillo-derived *M. leprae*. We made some *M. leprae* suspensions from the tissues of nine-banded armadillos. These animals were experimentally inoculated intravenously with 1×10^9 nude mouse-derived leprosy bacilli and had incubated the infection for between 14 to 30 months before being harvested in the very latest stages of fully disseminated leprosy. Bacterial suspensions derived from armadillos were prepared as fresh tissue homogenates of highly bacilliferous liver, spleen, lymph node or leproma in a manner analogous to the procedure used for nude mouse foot pads described above. All of the armadillo tissues contained greater than 1×10^9 *M. leprae* per gram. The tissues were taken at varying times over a 3-year period and all were free of cultivable contaminants.

Radiorespirometry (RR)

We assessed the ability of each *M. leprae* suspension to oxidize ^{14}C-palmitate in BACTEC 7H12B medium (Becton Dickinson Inc., Franklin Lakes, New Jersey, U.S.A.). Suspensions of *M. leprae* were incubated in commercially prepared BACTEC vials containing 4 ml of the medium, or in closed-cap Buddemeyer-type culture vessels containing 1 ml of the same commercially prepared BACTEC medium. Oxidation of palmitate by *M. leprae* resulted in the release of radiolabeled CO_2. The evolved gas was detected either directly, in the case of BACTEC vials, with a BACTEC 460 instrument or indirectly, in the case of Buddemeyer vessels, on a Beckman LS6000ic (Beckman Counter, Inc., Fullerton, California, U.S.A.) scintillation counter with the aid of absorbent strips saturated with sodium hydroxide and scintillation fluid inside each Buddemeyer vessel ([4]). For BACTEC vials, an average growth index (GI) for each suspension was determined on triplicate vials inoculated with 1×10^7 bacilli after they had incubated for 7 days at 33°C. The mean GI was converted to DPM/10^6 bacilli using the manufacturer's formula accompanying each media lot. The activity in Buddemeyer vessels was read directly 7 days after those cultures had incubated at 33°C. For comparative purposes, the mean DPM/10^6 bacilli were derived from triplicate sets.

Mouse foot pad studies

The level of growth that each *M. leprae* suspension achieved in conventional mouse foot pads was considered the standard for viability. The MFP technique was performed according to the method described by Shepard with minor modifications ([15, 19]). In brief, 30 µl aliquots of bacillary suspensions containing 1×10^4 *M. leprae* diluted in Hanks balanced salt solution (HBSS; Gibco, Grand Island, New York, U.S.A.) were inoculated into both hind foot pads of conventional BALB/c mice (Harlan). The foot pads were harvested at 90, 120, or 150 days post-inoculation in order to assess the level of growth achieved by the bacillary suspensions. In experiments where mice were harvested at multiple time intervals, control samples of freshly harvested *M. leprae* diluted to concentrations of 1×10^4, 1×10^3 and 1×10^2 bacilli in 30 µl HBSS also were inoculated into mouse foot pads. These diluted control samples were used to simulate the growth that might be expected after a –90% and –99% effective loss of viability in those individual preparations ([9]). For enumeration of MFP growth, four mice were harvested in each group with counts made on paired samples and the observations averaged.

Storage and handling conditions

We compared the ability of several *M. leprae* suspensions to maintain viability during storage. After harvesting *M. leprae* from nude mouse foot pads, bacilli were aliquoted to 20 ml closed-cap serum vials (Wheaton) containing 4 ml of Middlebrook 7H12 at a concentration of 1×10^8 *M. leprae*/ml. Immediately after dispensing the

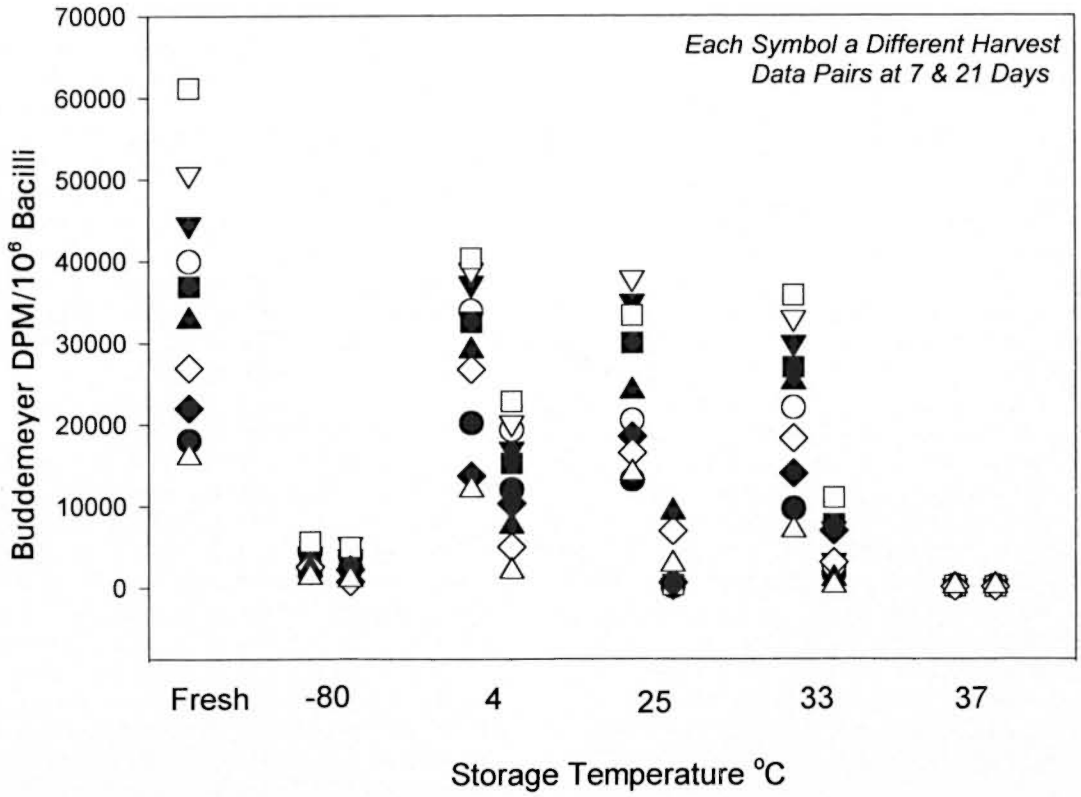

FIG. 1. Scattergrams depicting the radiorespirometric activity of different *M. leprae* suspensions (N = 10) upon initial harvest from nude mouse foot pads (fresh) and after storage in 7H12 liquid medium at temperatures ranging from 0°C to 37°C. Each harvest is represented by its own symbol. Paired scatters at each temperature demonstrate the activity of that preparation with storage for 7 days and 21 days, respectively. Figure shows that *M. leprae* vary in activity with each harvest and viability wanes with storage.

bacilli to storage vials, aliquots of the sample were removed to determine its original baseline activity using RR and MFP. Afterward, the vials were held at –80°C, 4°C, 25°C, 33°C, and 37°C for up to 3 weeks. Additional aliquots of identical amounts of bacilli were removed again at 7- and 21-day intervals to retest the samples for changes in viability during storage. The RR and MFP results derived from individual stored samples were compared to the baseline results originally obtained with each fresh suspension and expressed as a percent of the original activity. Some RR and MFP results also were correlated directly in a linear regression model to discern the association of palmitate oxidation with MFP growth. All statistical comparisons were made on a personal computer using the SAS program package (Statistical Analysis Systems, Cary, North Carolina, U.S.A.).

RESULTS

Maintenance of metabolic activity with storage

Suspensions of *M. leprae* prepared from different nude mouse foot pads are not uniform in their ability to oxidize ^{14}C-palmitate, but exhibit a broad range of detectable metabolic activities. The original activity tends to wane over time. We used 10 different suspensions to examine the ability of *M. leprae* to maintain their metabolic activity when stored for periods of 1 to 3 weeks in 7H12 liquid medium at temperatures ranging from –80°C to 37°C. As shown in Figure 1, the oxidative metabolism of palmi-

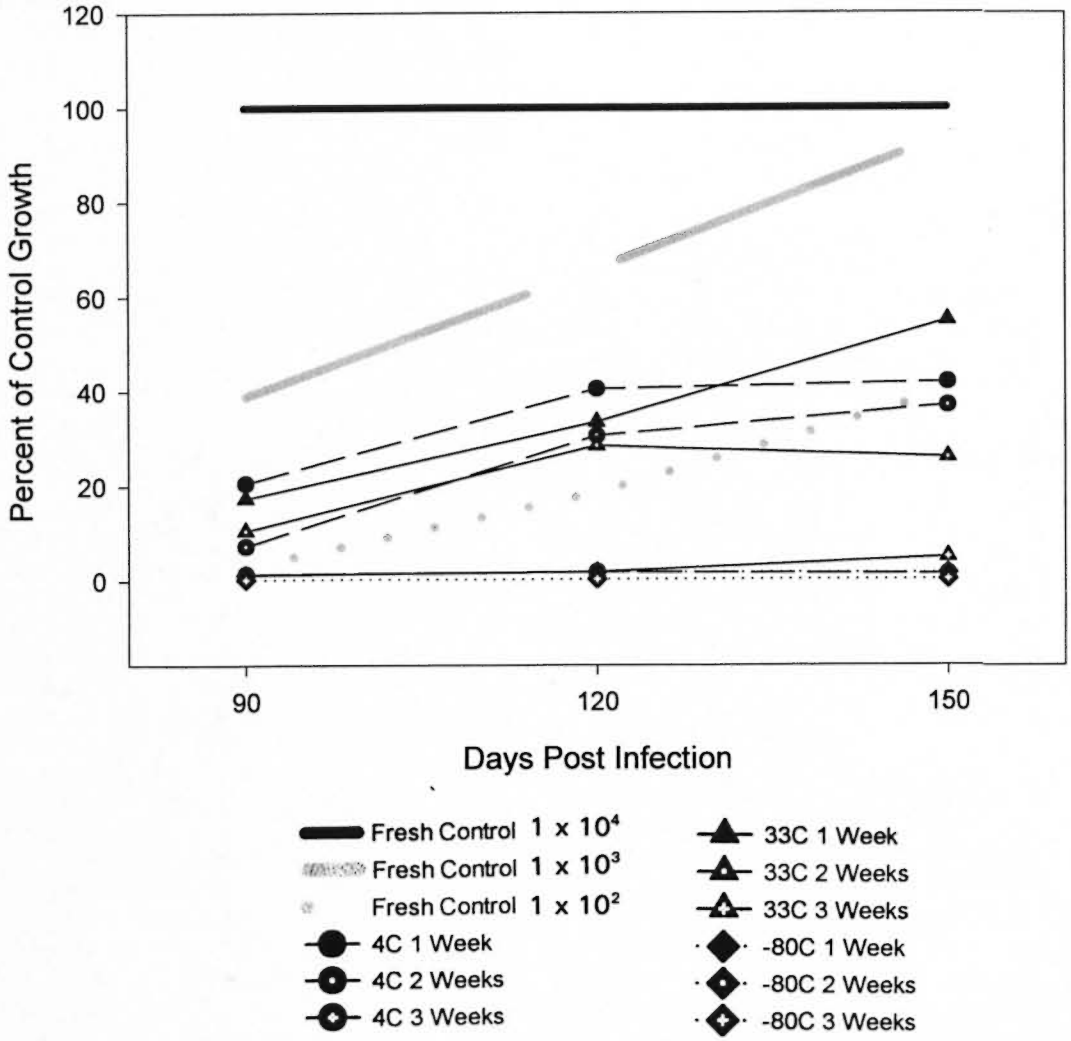

FIG. 2. Average percent of growth in conventional mouse foot pads at various time intervals achieved by *M. leprae* suspensions (N = 3) stored in 7H12 liquid medium at –80°C, 4°C and 33°C for 7, 14 and 21 days as compared to the baseline growth achieved by the same fresh bacillary suspensions prior to storage. Fresh control samples were inoculated at full strength (10^4/foot pad) and at concentrations simulating a –90% and –99% effective loss in viability (10^3/foot pad and 10^2/foot pad, respectively). Stored samples failed to grow to the same levels as full strength fresh samples.

tate declined markedly in each sample over the 3 weeks of storage as compared to the activity originally detected in the fresh suspension. Regardless of the storage conditions used, most samples retained <10% of their original activity within 3 weeks.

The oxidative activity of *M. leprae* was preserved best by suspensions held at 4°C, 25°C and 33°C. On average, suspensions held at 4°C retained about 60% of their oxidative activity after 1 week. That activity waned markedly during additional time in storage. Suspensions held at 25°C and 33°C also tended to maintain higher metabolic activities than suspensions held at other temperatures, but those samples also showed a marked decline in oxidative activity during the 3 weeks in storage. Suspensions held at 33°C lost an average of 20% of their metabolic activity within 1 week,

and 60% of their overall activity in 3 weeks. Suspensions held at 25°C showed a similar pattern of decline. The percent of loss tended to be greatest among suspensions whose fresh samples had the highest original baseline activities.

It is especially noteworthy that in each of the replicate experiments, *M. leprae* suspensions held at 37°C, or subjected to freeze-thaw cycles, showed very rapid loss of metabolic activity. Virtually all of the detectable activity toward palmitate was lost within only a few days when *M. leprae* cultures were incubated at 37°C. Bacillary suspensions held at –80°C and thawed once also registered only 1%–5% of the metabolic activity detected in their fresh suspensions. Repeated freeze-thaw cycles eliminated virtually all of their detectable oxidative activity. In addition, retaining the bacilli in whole tissues during freezing did little to preserve their metabolic activity. Bacillary suspensions prepared from four different 0.5-g nude mouse tissue samples frozen to –80°C for 24 hr retained an average of only 7% ± 3.6% of the oxidative activity exhibited by bacillary suspensions prepared from portions of the same tissues before freezing.

Loss of infectivity with time and temperature

We also examined the influence of storage on *M. leprae* viability as determined with the conventional MFP technique. Bacillary suspensions derived from three different nude mice were held from 0 to 3 weeks at –80°C, 4°C and 33°C before aliquots were removed and tested for viability. As shown in Figure 2, stored suspensions lost viability quickly and failed to grow in the mouse foot pads to the same levels as the full-strength, fresh control samples.

In comparing the level of growth achieved by stored suspensions to control samples at various time points, we can estimate the relative loss in viability incurred during storage. Bacillary suspensions held in storage at either 4°C or 33°C for up to 2 weeks grew in MFP to levels generally comparable to fresh suspensions which contained only 10% of the number of viable bacilli. Samples which had been frozen to –80°C and thawed even once or repeated times showed either no detectable growth in MFP at all or grew to levels comparable to fresh bacillary suspension controls which contained only <1% of the viable bacilli as the full-strength fresh sample. Regardless of the storage temperature used, suspensions held for 3 weeks grew poorly in MFP and failed to achieve growth levels comparable to control inocula, which contained <1% of the viable bacilli as the original full-strength fresh samples.

Correlation of MFP and RR

To discern the association between oxidative metabolism of ^{14}C-palmitate and the actual viability of *M. leprae* suspensions, we compared RR data with the 120-day MFP result in a linear regression model. We used the values obtained from suspensions stored for up to 2 weeks in the previous storage experiment, along with data from 10 additional *M. leprae* harvests which we prepared from other nude mice in our laboratory during the same time period (total N = 47). As shown in Figure 3, values for the oxidative metabolism of ^{14}C-palmitate among the different *M. leprae* suspensions were highly correlated with the level of growth they achieved in the MFP ($r = 0.71$). Although the RR and MFP results were derived independently over a period of several months, the majority of the observations fell well within the 95% confidence interval inscribed around the linear regression line. The relative level of palmitate oxidation by *M. leprae* suspensions appears to be highly proportional to the growth they can achieve in the MFP at the 120-day interval.

Viability of *M. leprae* obtained from different tissues

To better understand parameters for *in vivo* propagation likely associated with harvesting highly viable *M. leprae*, we examined the oxidative activity of bacilli derived from a number of different nude mouse foot pads and armadillo tissues. As shown in Figure 4, the average metabolic activity for *M. leprae* from the different armadillo tissues tended to be lower than the activity of bacilli harvested from nude mice. The activity was lowest among bacilli derived from armadillo liver, and was only moderately higher for bacilli obtained from armadillo spleen and lymph node tissues. The

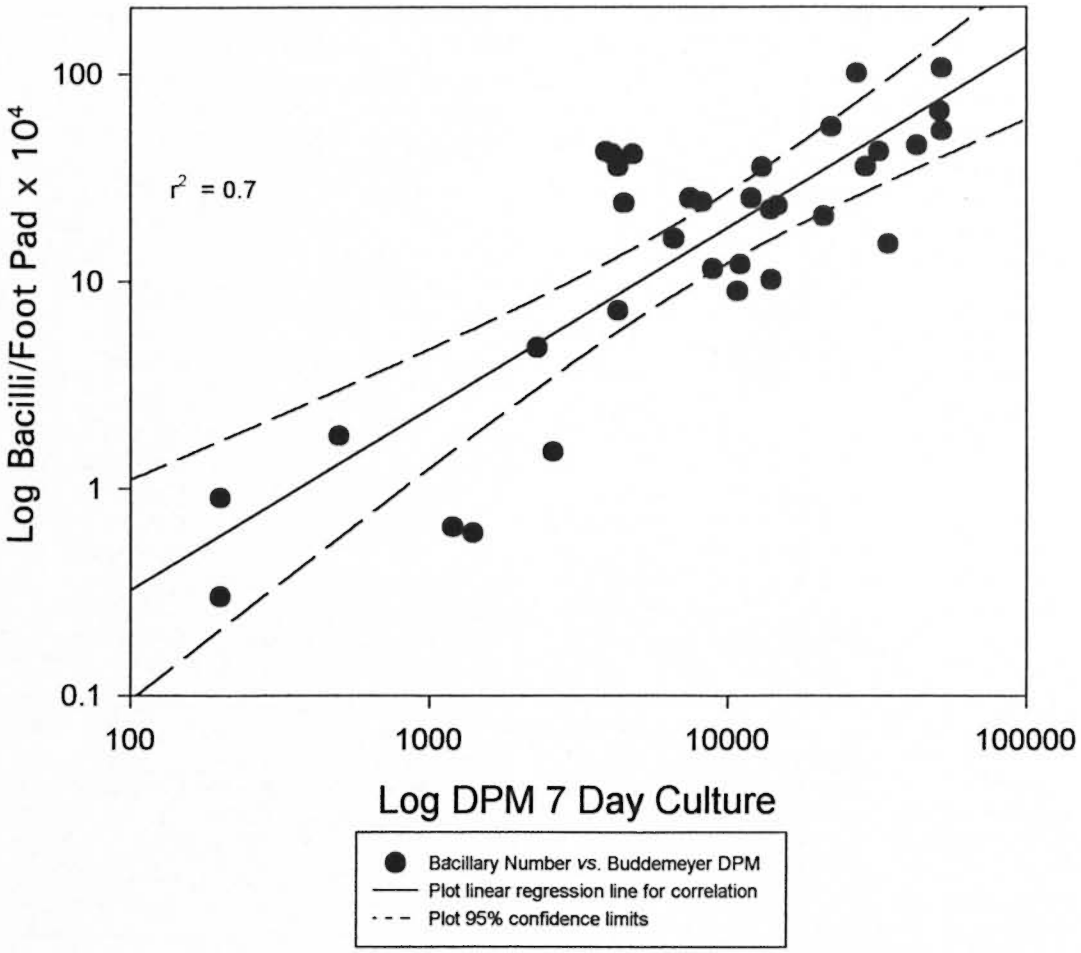

FIG. 3. Linear regression model comparing radiorespirometric activity of *M. leprae* suspensions (N = 47) with conventional MFP growth results obtained for each suspension at 120 days postinfection in the mouse (r = 0.71). The data in each axis are log transformed.

average activity for bacilli from armadillo leproma was not significantly different from the other armadillo tissues. However, leproma-derived bacilli also showed the greatest variability in oxidative activity of all the types of tissues tested.

The oxidative activity observed among nude mouse-derived bacilli was significantly higher than that from armadillos. However, it too showed considerable variability. The nude mouse foot pads we harvested were mostly of medium size, averaging 0.82 g, but data was available on foot pads ranging in size from 4.1 g to 0.1 g. We compared the RR results from these different harvests with the duration of each infection in the mouse and its bacillary yield. As shown in Figure 5, the highest metabolic activities were associated with shorter intervals of infection that yielded low-to-moderate numbers of *M. leprae*. Bacilli from very large, especially ulcerated foot pads, showed the lowest overall activity. The three-dimensional figure depicts the association of increasing time and bacillary number with declining radiorespirometric activity. No data were available for infections of less than 140 days' duration, and the relationship of metabolic activity or viability with very short-term infections in nude mice is not described. However, with increasing duration of infection or bacterial number the viability of *M. leprae* obtained from nude mouse foot pads declines.

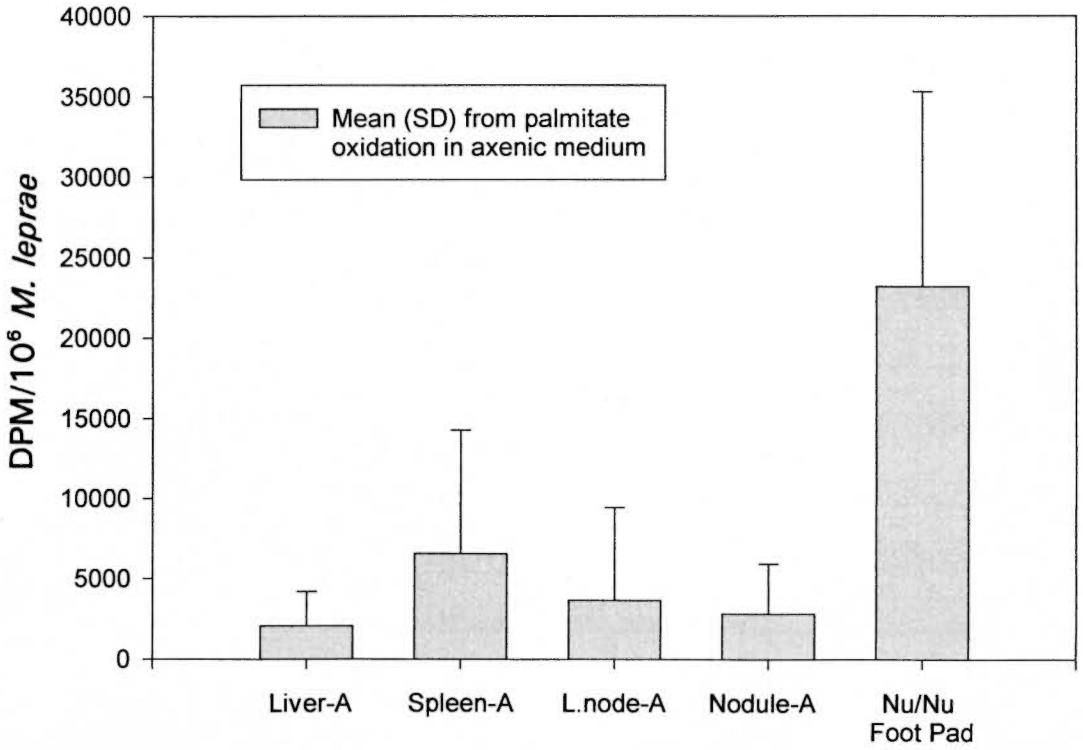

FIG. 4. Histogram depicting mean and standard deviation DPM/10^6 bacilli in palmitate oxidation assay of *M. leprae* derived from fresh samples of armadillo liver (N = 17), spleen (N = 16), lymph node (N = 11), leproma nodule (N = 5) and nude mouse foot pads (N = 63). The relative activity and viability of *M. leprae* vary widely between different hosts and tissues.

DISCUSSION

These data indicate that the oxidation of ^{14}C-palmitate by *M. leprae* in axenic culture can be used effectively as a rapid index of viability. The assay detects very subtle differences in the metabolic activity of *M. leprae*, which we have shown to be highly correlated with the growth that these bacilli achieve in conventional mouse foot pad (MFP) studies. Our findings also show that many of the storage conditions or incubation temperatures commonly used in the laboratory can rapidly, and markedly, affect the viability and metabolic capacity of freshly harvested *M. leprae* and can impact their potential use as a leprosy research resource. Most notably, a single freeze-thaw cycle or incubation at 37°C for just a few days has extremely deleterious effects. Modern laboratory studies addressing the molecular regulation of *M. leprae*, its secreted antigens or the differential host response to living and dead leprosy bacilli require the use of highly viable *M. leprae*. Palmitate oxidation assays can provide a convenient means to assess viability of *M. leprae* and can be used effectively to aid in the design of laboratory protocols which benefit survival and maintenance of metabolic activity by the organisms under study.

M. leprae does not multiply in axenic culture and propagation of bacilli in the MFP has been the only established means to evaluate viability. The procedure is time-consuming and difficult. The time to first appearance of bacterial growth and its eventual plateau in the MFP is known to be a function of the proportion of viable organisms in the inoculum ([9]). The differential growth results seen here in the MFP for the various inocula tested are caused by differences in the proportions of viable organisms in those samples. The relative number

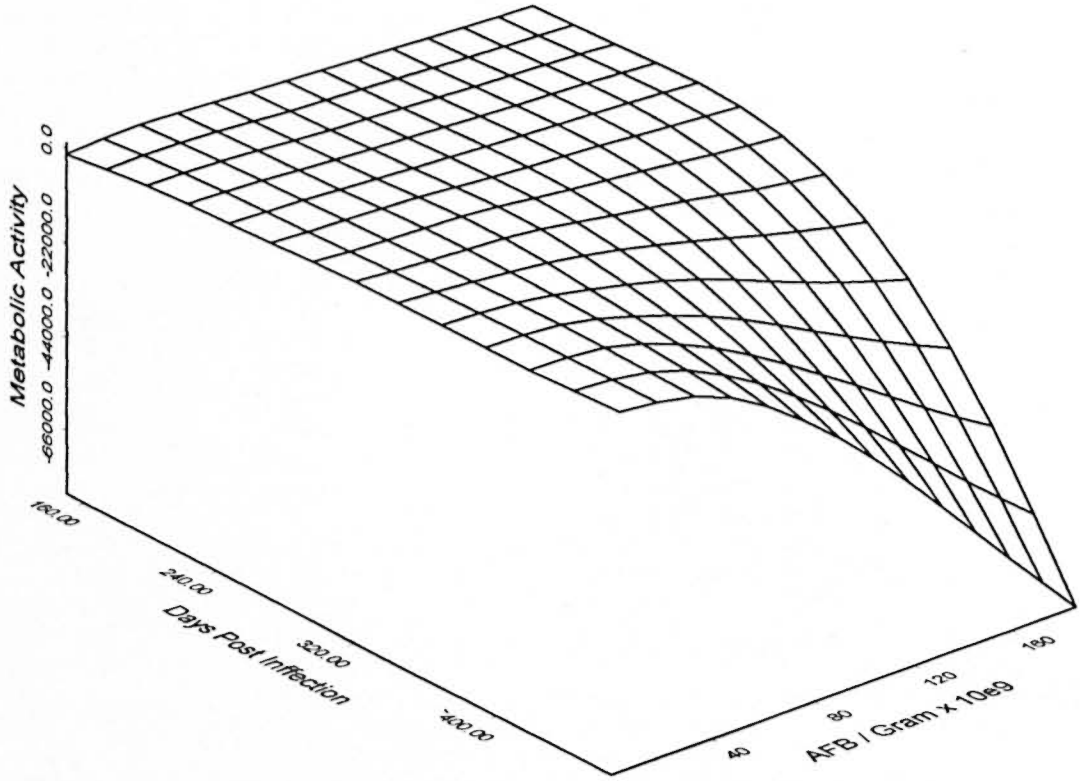

FIG. 5. The three-dimensional relationship between the bacterial number (AFB/gram) and duration of infection (days postinfection) with the relative viability of *M. leprae* harvested from nude mouse foot pads (N = 63) as expressed by their metabolic activity in oxidation of ^{14}C-palmitate in axenic culture. The declination in metabolic activity describes decreasing *M. leprae* viability in large nude mouse foot pads in late-stage infections.

of viable *M. leprae* contained in different bacillary preparations vary markedly between donor hosts, the type of tissue, and characteristics of the individual infection, as well as the age of the suspension and conditions under which the bacilli are stored and used.

The high statistical correlation seen here between the RR values and MFP growth results of bacillary suspensions confirm the quantitative nature of palmitate oxidation for indexing *M. leprae* viability. These observations were made on a large number of samples processed over many different days. The degree of correlation expressed between MFP and RR probably was influenced by inter-run variations and other errors in the reproducibility of both assays, especially the MFP procedure. Palmitate oxidation actually may reflect more subtle differences in *M. leprae* viability than can be detected in the conventional MFP technique. Since the assay describes the relative viability of a suspension at a specific point in time, tests run at multiple time points would likely be useful for describing trends in viability and could likely increase sensitivity of the technique overall.

A number of methods have been employed as surrogates to MFP testing. *M. leprae* in skin biopsies become granular after patients initiate drug therapy, and such morphologic changes have been used to estimate qualitative differences in the viability of *M. leprae* preparations based on their morphologic index (MI = percent of solid-staining normal organisms in a microscopic field) ([11, 20]). Unfortunately, the MI is not reproducible between laboratories and tends to vary between individual runs, technicians and the staining reagents used. So-called viable stains, such as FDA-EB, appear to have similar problems in reproducibility between laboratories and in

association with MFP results ([8, 13, 18]). In addition, FDA-EB staining has not proven to be effective for *in vitro* drug susceptibility testing, and *M. leprae* may require *in vivo* incubation before viability differences can be noted with this procedure.

Other biochemical assays also may be useful for indexing *M. leprae* viability. Some studies have suggested that the relative concentrations of ATP among *M. leprae* is broadly associable with the likelihood that they may eventually manifest detectable growth in the MFP. However, the specific cellular concentration of ATP required has not been standardized, and the assay is not known to discern subtle differences in viability ([3, 6]). Similar to palmitate, the uptake of hypoxanthine by *M. leprae* in axenic culture is reported to be useful for *in vitro* drug screening. However, its use as a quantitative index of viability was discounted early on because hypoxanthine incorporation appeared to increase continuously even among relatively old *M. leprae* suspensions with declining viability ([21]). Palmitate oxidation assays show a broader range of activity than hypoxanthine and require considerably fewer bacilli (10^{5-6} compared to 10^{8}). However, some culture systems also can erroneously suggest that palmitate is metabolized continuously by aging *M. leprae* suspensions.

Oxidation of ^{14}C-palmitate in axenic culture results in the evolution of radiolabeled CO_2, which can be detected in a number of different ways. When using the BACTEC 460 instrument, the respiration index for *M. leprae* in culture rises rapidly during the first 7 days to a plateau which is sustained for another 2 weeks. However, the actual viability of *M. leprae* in these aging cultures continues to decline. Therefore, the third week GI of BACTEC cultures correlates very poorly with the MFP results of *M. leprae* suspensions of this age. Interestingly though, when those stored *M. leprae* suspensions are subcultured they show a similar activity pattern in BACTEC; rising rapidly for 7 days to a plateau that is sustained for another 2 weeks. The peak GI achieved by those aged subcultures, however, is markedly lower than the peak achieved by their fresh counterparts. The lower peak of the stored cultures is consistent with the lower viability of the aged organisms. With BACTEC, the peak GI is highly correlated with the suspension's MFP growth results. The second and third week plateau GIs appear to be just an artifact of the BACTEC 460 culture system. Buddemeyer-type (biphasic) culture systems also show a 7-day peak in oxidative metabolism of ^{14}C-palmitate by *M. leprae.* BACTEC and Buddemeyer RR results are highly correlated, both individually and with MFP. Therefore, the rate that a substrate is metabolized by *M. leprae* in axenic culture may be far more important in terms of viability than its apparent duration or total uptake.

Supplies of *M. leprae* used in experimental studies are usually propagated in either nude mice or armadillos. These hosts serve different roles in propagation and the leprosy bacilli become manifest in their tissues by different routes. Nude mouse-derived *M. leprae* tend to show higher RR activity than *M. leprae* obtained from armadillo tissues. Armadillos yield hundreds of times more *M. leprae* than can be derived from a nude mouse, and they are harvested only after a long incubation period in the latest stages of experimental leprosy in order to obtain those maximum bacillary yields. *M. leprae* infections of nude mice are more easily scheduled and controlled, and nude mice can be a good source for small quantities of highly viable *M. leprae.* However, the viability of nude mouse-derived *M. leprae* also varies markedly. *M. leprae* taken from nude mice in very late stages of experimental infection or derived from very large or ulcerated foot pads also show low viability. Therefore, the intended use of the bacilli really dictates what propagation method is most appropriate.

The quality of viable *M. leprae* as a research reagent is markedly influenced by storage conditions and incubation temperatures. We and a number of other investigators have observed that without regard to the storage conditions used, *M. leprae* suspensions lose nearly all of their detectable metabolic activity and a significant proportion of their total viability within 3 weeks of harvest from host tissues. A similar degree of loss is incurred within just hours if the bacilli are incubated at 37°C or frozen even a single time ([2, 10, 12, 16, 17]). These findings can bring into question the conclusions

drawn in many scientific studies which used so-called "viable" *M. leprae* derived from frozen stocks, or incubated the bacilli in experiments at temperatures clearly detrimental to maintenance of *M. leprae* viability. The optimal temperature for *in vivo* growth of *M. leprae* has been shown to be 33°C ([14]). Viable *M. leprae* must be used soon after their harvest from host tissue and are best when stored for only short periods of time at 4°C.

We currently share viable *M. leprae* with other laboratories by shipping freshly harvested bacillary suspensions on wet ice via express courier. The delivery range is limited and this is a cumbersome method of supply. The leprosy bacilli remain usable for only for a short period of time, and better preservation and handling methods are needed. Freezing *M. leprae* in whole tissues appears to have little benefit for the maintenance of their viability. Preliminary results in our laboratory suggest that program freezing bacilli in whole organs or in dimethyl sulfoxide (DMSO) is somewhat superior to snap freezing bacillary suspensions without protectants, but only marginally so (unpublished observations). The potential utility for modern cryoprotectants or more innovative program freezing protocols that might improve maintenance of *M. leprae* viability during storage or shipment still merits additional investigation.

M. leprae have been isolated from dust, droplets, moist soil and dried sputum ([7]). Observations on the transient viability of *M. leprae* in the laboratory always seem to contrast with reports of recovering *M. leprae* from harsh conditions in the natural environment. However, survival of bacilli should not be confused with maintenance of high viability. Reports on recovery of leprosy-like bacilli from the environment are based largely on detecting small quantities of acid-fast organisms in the MFP—either on initial isolation or in successive blind passage. Relatively few *M. leprae* are needed to infect the MFP ([11]), and the number of viable organisms that might be required for growth to become detectable or to eventually reach plateau levels in the MFP can vary by tens of thousands ([9]). Since lepromatous hosts are known to shed large numbers of leprosy bacilli ([7]), survival and occasional recovery of fractional numbers of organisms from the natural environment is not really inconsistent with the highly transient viability of *M. leprae* described here.

Palmitate oxidation is a convenient, easy to perform assay. The substrate is used rapidly and reliable activity is detectable with as few as 10^{5-6} *M. leprae*. The reagents are generally available commercially, but assay results will vary with different media components and culture conditions. The relative amount of $^{14}CO_2$ derived from palmitate in axenic culture is influenced by the saturation of the label, competing carbon sources, temperature, the pH of the medium and the atmosphere of the culture chamber. Use of palmitate oxidation to index *M. leprae* viability in other laboratories will require standardization of reagents or revalidation of these test results. Nonetheless, oxidation of ^{14}C-palmitate is an objective reliable means to rapidly assess the viability of *M. leprae*, and the technique likely will benefit a number of studies in the future.

SUMMARY

Mycobacterium leprae remain a rare research resource. They cannot be cultivated on artificial media, and the only established means to quantify viability of *M. leprae* has been by its relative growth in the foot pads of conventional mice (MFP). The MFP method is technically difficult and requires several months to yield results. More effective methods are needed.

We examined the association between *M. leprae*'s ability to oxidize ^{14}C-palmitate in axenic culture and the MFP growth results of a large number of suspensions. Oxidative activity was assessed by radiorespirometry (RR) using the Buddemeyer-type biphasic culture vessels containing 7H12 liquid medium and ^{14}C-palmitate, or with commercially prepared BACTEC 12B vessels containing the same medium. The RR results were highly correlated ($r = 0.71$) with the growth level that each *M. leprae* suspension achieved by the MFP technique. In using this technique to examine the effects that many common laboratory practices have on *M. leprae* viability, we found that viability varies markedly between bacillary suspensions derived from different hosts and tissues. The highest viabilities were obtained with bacilli from moderately en-

larged nude MFP (<1 g). Viability tended to be lower among very large nude MFP or long-duration infections and from armadillo tissues. After their harvest from host tissues, leprosy bacilli lost viability quickly.

Suspensions stored in 7H12 liquid medium retained <1% of their viability within 3 weeks of harvest, and freezing bacillary preparations or incubating them at 37°C resulted in nearly an immediate equivalent loss in metabolic activity and viability. *M. leprae* viability is maintained best when bacilli are stored for only short periods of time at 4°C–33°C. Palmitate oxidation is a rapid, reliable and objective means by which to estimate the viability of *M. leprae* and can be used effectively as a surrogate for the conventional MFP technique in many studies.

RESUMEN

Mycobacterium leprae sigue siendo un raro espécimen de investígación. No se ha podido cultivar en medios de cultivo artificiales y el único medio establecido para cuantificar la viabilidad de *M. leprae* es su crecimiento relativo en la almohadilla plantar del ratón (APR). El método de la APR es técnicamente dificil y requiere varios meses para obtener resultados. Se necesitan métodos más efectivos.

En este trabajo, examinamos la asociación entre la habilidad de *M. leprae* para oxidar el ^{14}C-palmitato en un cultivo axénico y su capacidad de crecimiento en la APR. La oxidación del palmitato se estableció por radiorespirometría (RR) usando un sistema bifásico tipo Buddemeyer con medio 7H12 y ^{14}C-palmitato, o con el sistema comercial BACTEC con el mismo medio. Los resultados de la RR mostraron una alta correlación (r = 0.71) con el nivel de crecimiento de *M. leprae* en la APR. Usando esta técnica para examinar los efectos que tienen muchas prácticas de laboratorio sobre la viabilidad de *M. leprae*, encontramos que la viabilidad varía ampliamente entre las suspensiones de bacilos derivadas de diferentes huéspedes y tejidos. Las viabilidades más altas se obtuvieron con bacilos de las APR moderadamente engrosadas de ratones desnudos (<1 g). La viabilidad tendió a ser menor entre los bacilos de las APR muy engrosadas o en aquellas con mucho tiempo de infección y en las lesiones del tejido de armadillos. Después de su aislamiento de los tejidos infectados, el bacilo de la lepra perdió rápidamente su viabilidad.

Las suspensiones mantenidas en el medio líquido 7H12 retuvieron menos del 1% de su viabilidad a las 3 semanas de su aislamiento y el congelamiento de las suspensiones, o su incubación a 37°C, condujeron a la pérdida casi inmediata de su actividad metabólica y de su viabilidad. La viabilidad de *M. leprae* se preserva mejor cuando los bacilos se mantienen entre 4°C y 33°C por periodos cortos de tiempo. La oxidación del palmitato es una técnica rápida, corfiable y objetiva para determinar la viabilidad de *M. leprae* y puede usarse como una alternativa de la técnica de la APR en muchos estudios.

RÉSUMÉ

Mycobacterium leprae demeurent une ressource rare pour la recherche. Ils ne peuvent être cultivés sur milieu artificiel, et la seule méthode pour quantifier la viabilité se *M. leprae* a été de mesurer sa croissance relative dans la plante de patte de souris conventionnelles (PPS). La méthode PPS est techniquement difficile et demande plusieurs mois avant de pouvoir obtenir in résultat. Des méthodes plus efficaces seraient les bienvenues.

Nous avons examiné l'association entre la capacité de *M. leprae* à oxyder le ^{14}C-palmitate dans des cultures axéniques et les résultats d'un taux de croissance des PPS d'un grand nombre de suspensiones. L'activité oxydante fut évaluée par radio-respirométrie (RR) en utilisant le flaçon de culture biphasique de Buddemeyer contenant le milieu liquide de culture 7H12 et du ^{14}C-palmitate ou des flaçons BACTEC 12B préparés commercialement contenant le même milieu de culture. Les résultats de RR furent hautement corrélés (r = 0,71) avec les niveaux de croissance que chaque suspension de *M. leprae* a atteinte par la technique des PPS. Nous avons utilisé cette technique pour examiner l'impact sur la viabilité de *M. leprae* de quelques pratiques de laboratoire et nous avons constaté que la viabilité varie beaucoup entre des suspensions de bacilles provenant de tissus et d'hôtes différents. Les viabilités les plus élevées furent obtenues de suspensions bacillaires provenant de PPS de souries nues de taille moyenne (inférieure à 1 gramme). La viabilité tendait à être moindre parmi les PPS de grande à très grande taille ou celles avec une infection de longue durée et parmi les tissus infectés de tatous à neuf bandes. Après leur isolement à partir des tissus lésés de l'hôte, les bacilles lépreux pendent rapidement leur viabilité.

Les suspensions stockées 3 semaines dans le milieu de culture liquide 7H12 après collection des lépromes ont fortement perdu en viabilité, ne gardent que moins de 1% de leur viabilité initiale. La congélation ou l'incubation à 37°C de préparations bacillaires a entraîné une parte équivalente d'activité métabolique et de viabilité. Le meilieur moyen de conserver une bonne viabilité de *M. leprae* est de les conserver pendent une courte période entre 4 et 33°C. L'oxydation du palmitate est un moyen rapide, objectif et sûr d'estimer la viabilité de *M. leprae* et peut être utilisé comme méthode complémentaire à la technique PPS conventionnelle pour l'estimation de la viabilité dans de nombreuses études.

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