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# Respiration in *Mycobacterium leprae*[1]

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We ([15, 16, 18, 21]) have studied the respiratory enzymes and the terminal electron transport system in *Mycobacterium lepraemurium*, which are very difficult to cultivate, and have reported that *M. lepraemurium* lack cytochromes c and a. Wheeler, *et al.* ([27, 28]) have reported that *M. leprae* lacked catalase but had weak superoxide-dismutase activity. Kusunose, *et al.* ([10, 11]) have also reported that *M. leprae* had a fairly active superoxide-dismutase. It is very important to know the relationship between oxygen and the leprosy bacillus in order to attempt cell-free cultivation of *M. leprae*. Since the active oxygen which is produced in aerobic cultivation is very toxic to bacterial cells, it is important to know how the leprosy bacillus deals with this toxic oxygen. Every organism which lives in aerobic conditions has enzymes to degrade active oxygen ([3, 14]).

Purified leprosy bacilli can be collected easily and effectively from leprosy-infected armadillo liver by our Percoll gradient method ([22]) but, since infected armadillo liver is often stored in a frozen state for long periods of time, we thought that this material might not be suitable for measurements of respiratory enzyme activity. Therefore, we used fresh nude mouse lepromas as a source of the leprosy bacilli. We encountered an unexpected phenomenon, namely, that fresh homogenates of nude mouse foot pads agglutinated in the Percoll solution. Therefore, we devised a Ficoll gradient method to collect the bacilli from this source.

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## MATERIALS AND METHODS

### Preparation of bacilli

**Source.** In all, three isolates of *M. leprae* were used, the Thai 53 and Kurume Naha isolates in nude mouse foot pads and bacilli from armadillo liver.

**Purification.** An isolate of *M. leprae* designated Thai 53 was prepared to contain $10^7$ bacilli suspended in 30 $\mu$l of F12 tissue culture medium and inoculated into both hind foot pads of nude mice. The nude mice were maintained in vinyl isolaters, and lepromas developed in the inoculated foot pads after 16 months. Because of difficulty in obtaining homogeneous bacillary suspensions from these lepromas using conventional blender homogenization, we devised the following method of purifying the bacilli.

Starting with 3.6 g of foot pad lepromas (6 foot pads from three animals), the bones were separated from the soft connective tissue, separately minced with scissors, and thoroughly ground with a mortar and pestle. Soft tissue from 2 foot pads were used in each of three 15 cm diameter mortars, and bones from 3 foot pads were used in each of two additional mortars. The ground preparations were then allowed to stand at room temperature until dry. This step was found to be necessary to disperse globi.

Five ml of distilled water was gradually added to each mortar with grinding to make a bacillary suspension. The suspensions were then filtered through absorbent cotton into a small funnel. The mortars were rinsed with 5 ml of distilled water, and the washings filtered through the same absorbent cotton which was then carefully squeezed so that most of the fluid was expressed but all of the coarse tissue debris was retained. This filtrate was considered to be the original starting material for the purpose of calculating bacterial yields.

Discontinuous density gradient centrifugation of the filtrate was performed as shown in Figures 1, 2, and 3. Solutions of Ficoll

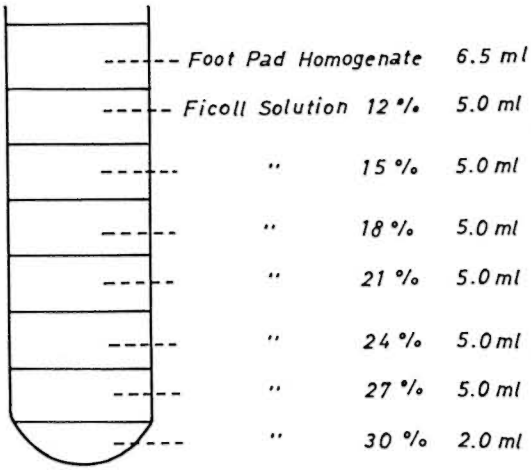


FIG. 1. Schematic of Ficoll 400 gradient preparation.

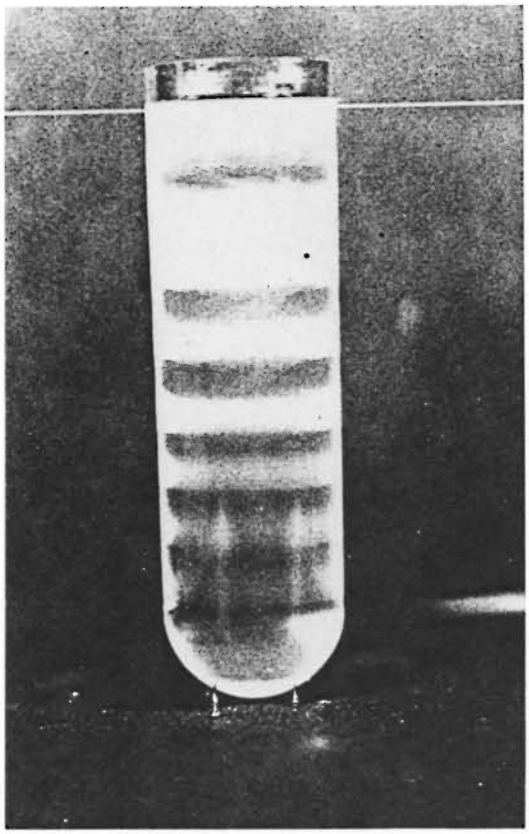

FIG. 2. Separation of leprosy bacilli in the Ficoll 400 density gradient centrifugation.

400 (Pharmacia Fine Chemicals, Uppsala, Sweden) ranging from 30% to 12% w/v in distilled water were successively overlaid in 38.5 ml volume Kontron LT cellulose centrifuge tubes, as indicated in Figure 1, and finally 6.5 ml of bacterial suspension was added. The tubes were then centrifuged at 100,000 × *g* for 60 min in a Beckman L8-55 ultracentrifuge in an SW 27 swinging bucket head. The bacilli were separated into 7 bands as shown in Figure 2. Each band was collected by puncturing the bottom of the centrifuge tubes with a needle and collecting the fractions dropwise. The fractions were designated 1 through 7 from the bottom of the tube, and were each washed twice with distilled water at 12,000 × *g* for 30 min. The final pellet of each fraction was used to calculate bacillary yields.

The pellets obtained above still contained tissue components. In order to eliminate them, the pellets were resuspended in 4% w/v NaOH, diluted eightfold in distilled water, and centrifuged at 12,000 × *g* for 20 min. The resulting pellet was washed with M/15 Sorensen phosphate buffer, pH 6.8, and the pellet was used as purified leprosy bacilli for the biochemical studies.

**Preparation of particle fraction.** The leprosy bacilli were disrupted by grinding in a mortar and pestle with quartz sand in a cold room with the addition of 0.25 M sucrose, M/75 phosphate buffer, pH 6.8 (phosphate buffered sucrose). The ground extract was clarified by centrifugation at 9000 × *g* for 60 min. The supernatant was then centrifuged in a Beckman Model L ultracentrifuge in a #40 head at 100,000 × *g* for 60 min. The pellet was used as a particle fraction.

## Biochemical studies

**Endogenous respiration.** The Warburg manometric method was used to determine the average coefficient of respiration ($QO_2$) of the bacilli.

**NAD-peroxidase.** Three ml of the clarified ground extract of the bacilli in phosphate buffered sucrose was added to a 10 mm quartz cuvette. An identical cuvette was filled with 3 ml of buffer alone. One-tenth ml of NADH (3.3 mg/ml) in the same buffer was added to both cuvettes, and the optical densities determined at 340 nm. One-tenth ml (2.5 μmol) hydrogen peroxide in water was then added, and the decreases in optical density at 340 nm monitored in a Hitachi Perkin-Elmer 139 spectrophotometer.

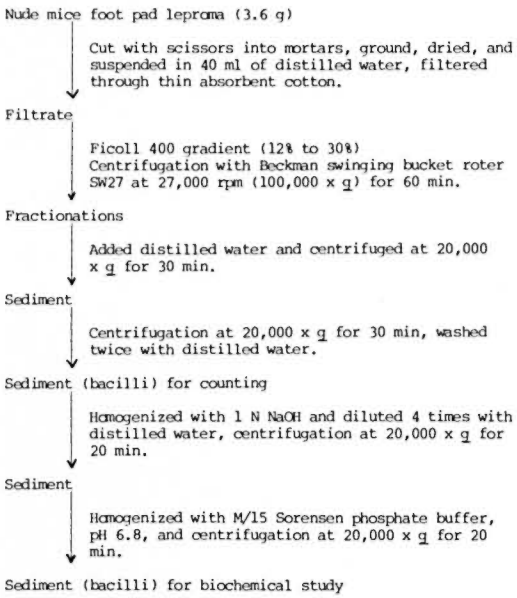


FIG. 3. Method of purification.

**Catalase activity.** This was determined by the sensitive method of Andreae ([1]) and Sorgato ([25]), based on the reduction in the fluorescence intensity of scopoletin upon oxidation by peroxidase. Hydrogen peroxide, 0.384 μmol/ml in water, and either the purified bacillary suspension or the cell-free extract in phosphate buffered sucrose were mixed in equal volumes. After incubation, 1 ml of the cell-free extract mixture or 1 ml of the supernatant after the mixture containing bacilli was centrifuged at 7000 × *g* for 3 min, was added to a previously prepared assay system. The assay system consisted of a mixture of 2 ml of scopoletin (Fulca, Buchs, Switzerland), 0.1 μmol/ml in 0.25 M sucrose, 10 mM Tris HCl buffer, pH 7.4, and 1 ml horseradish peroxidase (Sigma Chemical Corp., St. Louis, Missouri, U.S.A.), 400 μg/ml in the same buffer. One ml of the reaction mixture was added to the assay system and incubated for 30 min at 37°C. After oxidation of the scopoletin was complete, the remaining fluorescence intensity of the scopoletin was measured in an Aminco-Bowman fluorophotometer with excitation and emission wavelengths of 366 nm and 456 nm, respectively.

**Detection of cytochromes.** The oxido-reductive difference spectrum of cytochrome was determined with a Union Giken automatic scan spectrophotometer. Three ml volumes of bacterial suspension were added to two 10 mm quartz cuvettes. A few grains of hydrosulfite were added to one of the cuvettes, and the difference spectrum determined in comparison with the untreated control bacterial suspension.

TABLE 1. *Yields of* M. leprae *from leprosy-infected nude mouse foot pads.*

| | No. bacilli $\times 10^{10}$ | Yield (%) |
|---|---|---|
| Original homogenate | 54.0 | 100.0 |
| Fraction | | |
| 1 | 1.9 | 3.5 |
| 2 | 2.0 | 3.7 |
| 3 | 3.1 | 5.7 |
| 4 | 2.0 | 3.7 |
| 5 | 3.0 | 5.6 |
| 6 | 9.7 | 18.0 |
| 7 | 1.3 | 2.4 |
| Total | | 42.6 |

**Dehydrogenase activity.** One ml of cell-free bacterial extract in phosphate buffered sucrose was added to the main chamber of a Thunberg tube together with 0.1 ml of substrate, $10^{-5}$ mol/ml, and 0.1 ml of NAD (Wako Pure Chemical Corp., Osaka, Japan), $10^{-6}$ mol/ml. One-tenth ml of the electron acceptor, triphenyltetrazolium chloride (TTC), $5 \times 10^{-5}$ mol/ml in water was added to the side chamber of the tubes. After pressure in the Thunberg tube was reduced by vacuum, the contents of the main chamber and side chamber were mixed and incubated at 37°C for 2 hr. The enzyme reaction was stopped by adding 0.5 ml of 5% trichloroacetic acid, and the formazan which was produced in the reaction was extracted in 5 ml of ethyl acetate. The optical density of the red color of the formazan was determined at 480 nm. In the case of succinate dehydrogenase, the reaction mixture was supplemented with 0.1 ml of phenazine metasulfate (Sigma) $5 \times 10^{-6}$ mol/ml as an intermediate electron acceptor.

**AMPase activity.** The reaction mixture consisted of 0.5 ml of bacterial suspension; 0.2 ml of 0.1 M Tris buffer, pH 7.0; 0.1 ml of 0.01 N $MgCl_2$; 0.1 ml of 0.15 N KCl and 0.1 ml containing 6 μmol of the substrate, AMP (Wako Pure Chemical Corp.), in a total

TABLE 2. *Solid ratio in each fraction of leprosy bacilli.*

| Fraction | % Solid | % Nonsolid |
|---|---|---|
| 1–3 | 38 | 62 |
| 4 | 42 | 58 |
| 5 | 30 | 70 |
| 6 | 23 | 77 |

volume of 1.0 ml. The reaction mixture was incubated at 37°C for 60 min, and the reaction stopped with 0.5 ml of 3% perchloric acid followed by centrifugation. The inorganic phosphate liberated into the supernatant was determined by the method of Lowry and Lopez ([12]).

**Protein.** Protein was determined by the phenol method ([2, 13]).

## RESULTS

### Purification of the bacilli

Using the methods outlined in Figures 1, 2, and 3, seven fractions of leprosy bacilli were obtained. Dense particles of tissue, probably including DNA and RNA fragments, sediment to the bottom of the centrifuge tube in this procedure, entrapping a small number of bacilli. The yields of bacilli in each of these fractions are given in Table 1. Although the available counting methods for bacilli are imprecise, roughly 43% of the bacilli could be recovered with these methods. Leprosy bacilli have heterogeneous densities and, in general, are less dense than cultivable mycobacteria.

The percentage of the bacilli which stain solidly in each of the fractions is given in Table 2. Fraction 6 contained more nonsolid bacilli than did fraction 1 or fraction 5.

Mori, *et al.* ([20]) reported that the detection of AMPase activity in preparations of murine leprosy bacilli was a more sensitive measure of host-tissue contaminants than microscopic detection of blue-stainable tissue components. Accordingly, AMPase activity was measured in each of the fractions of bacilli in the present study and is given in Table 3. As may be seen, we were unable to obtain a fraction which was free from tissue components as judged by AMPase activity, or by staining (data not shown). Accordingly, we used alkali-treated bacilli, in which no AMPase activity was detectable, for the biochemical studies.

TABLE 3. *AMPase activity of separated* M. leprae *fractions.*

| | Dry weights mg of bacilli | Liberation nmol Pi | nmol Pi/mg |
|---|---|---|---|
| Original homogenate | 0.064[a] | 75 | 1172 |
| Nontreated fraction | | | |
| 1 | 0.54 | 38 | 70 |
| 2 | 0.24 | 13 | 54 |
| 3 | 0.32 | 25 | 78 |
| 4 | 0.38 | 43 | 113 |
| 5 | 0.46 | 114 | 248 |
| 6 | 0.53 | 100 | 187 |
| 7 | 0.65 | 135 | 208 |
| Alkali-treated fraction | | | |
| 1 | 0.40 | 0 | 0 |
| 2 | 0.13 | 0 | 0 |
| 3 | 0.23 | 0 | 0 |
| 4 | 0.21 | 0 | 0 |
| 5 | 0.12 | 0 | 0 |
| 6 | 0.34 | 0 | 0 |
| 7 | 0.36 | 0 | 0 |

[a] Protein.

### Biochemical studies

**Endogenous respiration.** As shown in Figure 4, oxygen consumption by leprosy bacilli proceeds linearly with time, therefore leprosy bacilli exhibit aerobic respiration. The average coefficient of respiration ($QO_2$) for the leprosy bacillus was 0.58 $\mu$l $O_2$/mg·hr compared with an average $QO_2$ of 4 $\mu$l $O_2$/mg·hr for the murine leprosy bacillus.

Fractions 1 through 5 were tentatively designated heavy bacilli and fractions 6 and 7 were termed light bacilli. Heavy and light fractions of leprosy bacilli were collected separately and compared as to their respiratory activities. As shown in Figure 5, the $QO_2$ of heavy bacilli was 0.31 $\mu$l $O_2$/mg·hr and was somewhat higher than the $QO_2$ of light bacilli which was 0.21 $\mu$l $O_2$/mg·hr.

**Cytochromes of *M. leprae*.** The oxido-reductive difference spectrum of the cytochromes of *M. leprae* is shown in Figure 6. The sharp Soret band of cyt $b_1$ at 426 nm and the peak of cyt $b_1$ at 560 nm are seen, but peaks at 550 nm and 630 nm corresponding to cyt c and cyt $a_2$ (the latter of which is seen in *M. lepraemurium*) were not detected in *M. leprae*. The same result was

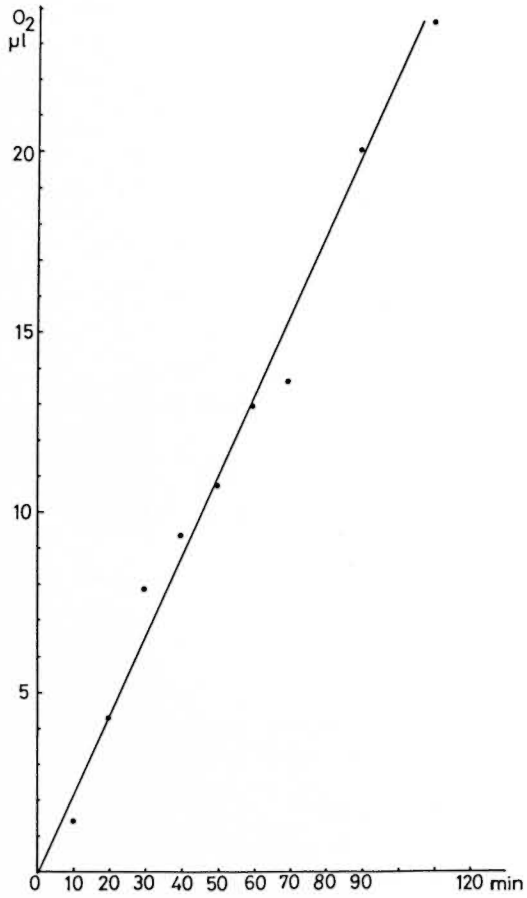


FIG. 4. Oxygen consumption in *M. leprae*. Dry weight of bacilli = 22.2 mg/2.5 ml vessel.

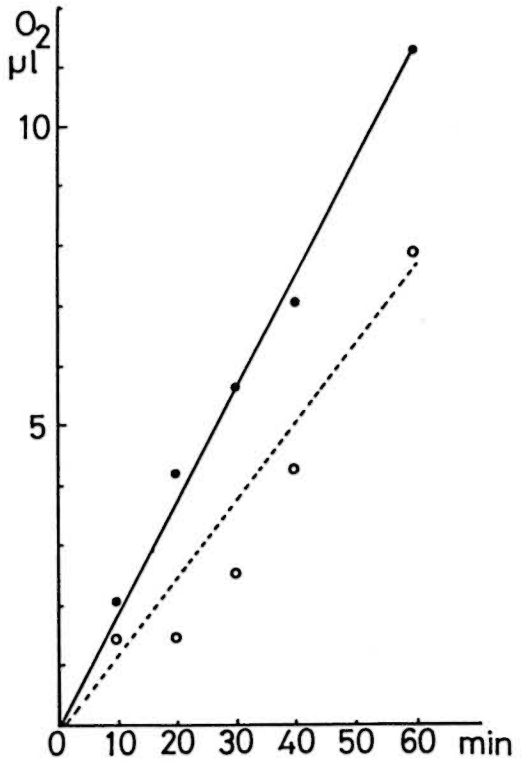


FIG. 5. Oxygen consumption in heavy (dense) and light (less dense) bacilli. ● = heavy bacilli (dry weight of bacilli = 36 mg/2.5 ml vessel); ○ = light bacilli (dry weight of bacilli = 38 mg/2.5 ml vessel).

obtained with leprosy bacilli harvested from infected armadillo liver and purified by the Percoll gradient method (Fig. 6).

In Figure 6, the area of the spectrum at 605 nm, corresponding to the expected peak for cyt a, was not clear. The cytochromes are concentrated in the particle fraction of the bacilli, therefore we examined the oxido-reductive difference spectrum of the particle fraction of the bacilli. As shown in Figure 7, the Soret peak at 426 nm and the peak at 560 nm corresponding to cyt $b_1$ are again seen, but the apparent change at 605 nm is seen equally well in the control baseline and appears to represent machine noise. Thus, cyt a was not detected in the particle fraction of *M. leprae*.

**Catalase activity in *M. leprae*.** The degree of inactivation of catalase by treatment with alkali was determined with *M. lepraemurium*. As shown in Figure 8, the catalase activity of alkali-treated *M. lepraemurium* was decreased to about one third that of untreated bacilli but was not completely abolished. With *M. leprae*, fairly strong catalase activity is seen with untreated bacilli but after alkali treatment the activity completely disappears.

Cell-free extracts of alkali-treated *M. leprae* and *M. lepraemurium* were then prepared and tested for catalase. The results are shown in Figure 9. *M. lepraemurium* showed fairly strong catalase activity but no activity was seen with cell-free extracts of *M. leprae*. Catalase activity in the particle fractions of alkali-treated *M. leprae* is given in Table 4 and all fractions were negative.

**NAD-peroxidase activity in *M. leprae*.** Mori, *et al.* ([17]) have reported that *M. lepraemurium* harvested from murine lepromas showed NAD-peroxidase activity as shown by the oxidation of NADH in the presence of hydrogen peroxide and a cell-

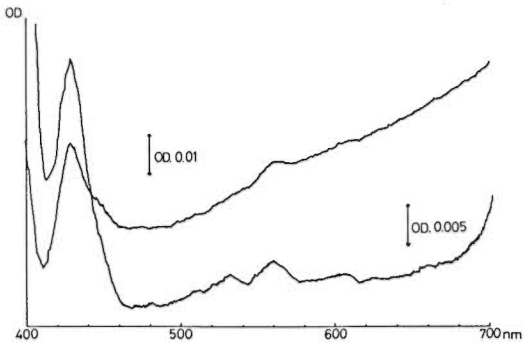


FIG. 6. Oxido-reductive difference spectrum of cytochromes in *M. leprae* Thai 53 and *M. leprae* collected from armadillo liver. OD 0.005 = *M. leprae* Thai 53 (dry weight of bacilli = 4.67 mg/ml); OD 0.01 = *M. leprae* from armadillo liver (dry weight of bacilli = 3.9 mg/ml).

free extract of the bacilli. The results of testing a cell-free extract of alkali-treated *M. leprae* revealed a lack of NAD-peroxidase activity (Fig. 10). Therefore, we conclude that NAD-peroxidase is not present in leprosy bacilli.

**Dehydrogenase activities in cell-free extracts of *M. leprae*.** As we have previously reported ([19, 21]), *M. lepraemurium* lacks glyceraldehyde-3-phosphate dehydrogenase, a key enzyme in the glycolysis system. Cell-free extracts of *M. leprae* were tested for dehydrogenase activities using the substrates glyceraldehyde-3-phosphate, α-glycerophosphate, glyceraldehyde, malate, succinate, and citrate. As shown in Table 5, *M.*

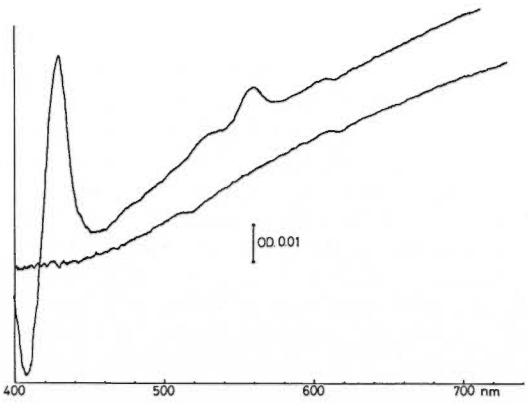


FIG. 7. Oxido-reductive difference spectrum of cytochrome in particle fraction of *M. leprae* Thai 53 strain. Protein content of particle = 1.2 mg/ml.

TABLE 4. *Catalase activity of particle fractions of* M. leprae *Thai 53 isolate.*

| | $H_2O_2$ | Scopoletin | Protein μg/ml | Fluorescence energy at 0.03 |
|---|---|---|---|---|
| — | + | + | — | 40 |
| — | — | + | — | 53,333 |
| Fr. 1–5 | + | + | 71 | 47 |
| Fr. 6–7 | + | + | 75 | 40 |
| Total Fr. | + | + | 65 | 43 |
| Total Fr. | + | + | 87 | 39 |

*leprae* showed malate dehydrogenase and succinate dehydrogenase activities, both being enzymes contained in the tricarboxylic acid cycle. Glyceraldehyde-3-phosphate dehydrogenase and α-glycerophosphate dehydrogenase activities were not found in *M. leprae*, both enzymes being part of the glycolysis enzyme system. Glyceraldehyde dehydrogenase activity was found with *M. leprae*, but the aldehyde dehydrogenase activity was general and not specific for glyceraldehyde. Citrate dehydrogenase activity, which is present in *M. lepraemurium*, was not found in cell-free extracts of *M. leprae*.

## DISCUSSION

Fresh, live leprosy bacilli may be necessary to optimally study biochemical enzyme activities in the organism. This presents a potential hazard to laboratory workers. The present method of collecting and purifying the bacilli offers the advantage of being safe from the point of view of infection with the live organism, results in only minor degradation of cell components, avoids the introduction of protease and DNAase, is simple, can be completed in one day, and provides good yields of the bacilli. A disadvantage of the procedure is that alkali treatment of the preparation is indispensable in obtaining pure bacilli. Alkali treatment is satisfactory for biochemical studies but may not be appropriate for vaccination or antigenic analysis studies of the bacillus because of degradation of surface proteins.

The leprosy bacilli were heterogeneous in density in the Ficoll gradient centrifugation. It may be that the more dense bacilli contain more DNA per cell than the less dense ones, the increased DNA being in preparation for

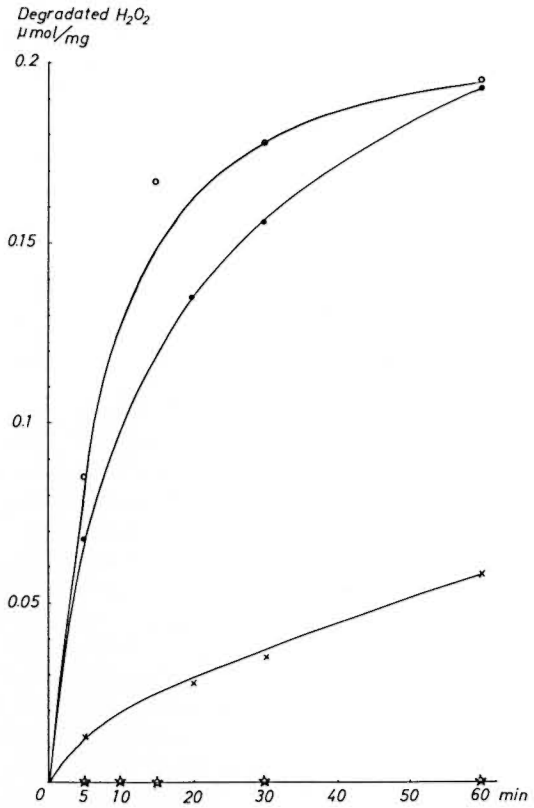


FIG. 8. Catalase activities in cultivated *M. lepraemurium* and *M. leprae*. ● = nontreated *M. lepraemurium*; X = alkali-treated *M. lepraemurium* (dry weight of bacilli = 0.48 mg/ml); ○ = nontreated *M. leprae* (dry weight of bacilli = 0.97 mg/ml); ☆ = alkali-treated *M. leprae* (dry weight of bacilli = 0.8 mg/ml).

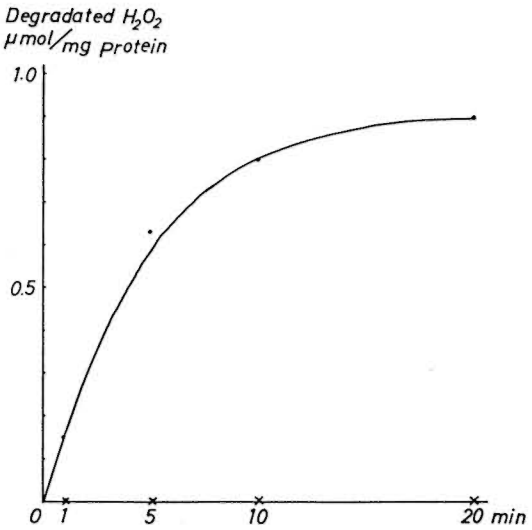


FIG. 9. Catalase activities of cell-free extracts from *M. leprae* and *M. lepraemurium*. ● = *M. lepraemurium* (133 μg protein/ml); X = *M. leprae* (196 μg protein/ml).

cell division. The less dense bacilli may have less DNA per cell and represent cells which have recently completed cell division. Some of the less dense cells may be partially degenerated, but substantial proportions of solid bacilli were also seen in these fractions. Imaeda [4] reported that leprosy bacilli have less DNA than other acid-fast bacilli (AFB). We tested cultivated *M. smegmatis* ATCC 23011, *M. avium* Kirchberg, *M. lepraemurium* Hawaiian, and *in vivo*-grown *M. lepraemurium* Hawaiian in the same Ficoll density gradient centrifugation system. We found that all were heterogeneous in density but that they were all more dense than *M. leprae,* almost all of them sedimenting to the bottom of the tubes. The rapid-growing *M. smegmatis* was the most dense of the species tested, perhaps due to the increased amount of DNA per cell as a result of the rapid rate of cell division.

**Respiratory enzyme system.** The collection method may be the most important procedure in studying the biochemical nature of the leprosy bacillus. Prabhakaran [23] and Ishaque and Kato [7] have reported on the biochemical properties of *M. leprae.* However, considering their collection methods, some armadillo tissue components may have remained in their bacterial fractions. Ishaque and Kato [7] reported that the cytochromes of *M. leprae* were cyt c, cyt b, and cyt $a_3$, but their results may depend on both cytochromes from the host tissue as well as the leprosy bacilli. Cultivated *M. smegmatis* ATCC 23011 and *M. avium* Kirchberg have cyt c, cyt $b_1$, and cyt a. However, *in vivo*-grown *M. avium* Kirchberg, collected from an infected mouse lung, had cyt $b_1$ and cyt $a_2$ but did not have cyt c or cyt a, i.e., it showed cytochromes similar to those found in *M. lepraemurium.* When *in vivo*-grown *M. avium* Kirchberg were isolated on 1% Ogawa medium, *in vitro*-grown *M. avium* produced cyt c and cyt a adaptively, but *M. lepraemurium* did not. Ishaque [5] detected cyt c in *M. lepraemurium* with the pyridine chemochromogen method. All of which confirms the DNA sequence coding for cyt c or cyt a.

TABLE 5. *Dehydrogenase activity of cell-free extract of* M. leprae *Thai 53 isolate (443 μg protein).*

| Substrates $10^{-5}$ mol | Formazan $10^{-7}$ mol |
|---|---|
| Malate | 6.7 |
| Succinate[a] | 10.2 |
| Citrate | 0 |
| Glyceraldehyde 3-P | 0 |
| α-Glycerophosphate | 0 |
| Glyceraldehyde | 6.1 |

[a] 153 μg Phenazine methosulfate.

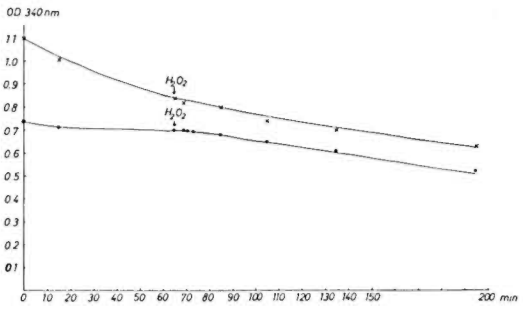


FIG. 10. NAD-Peroxidase activity in cell-free extract of *M. leprae*. X = NADH control; ● = cell-free extract of *M. leprae* (196 μg protein/ml).

The NADH oxidation enzyme system was not detected in *in vivo*-grown *M. lepraemurium*, but the enzyme was produced adaptively in cultivated *M. lepraemurium*. When *in vivo*-grown bacilli were transferred to medium, some enzymes were produced adaptively in the cultivated bacilli and some were not. If the leprosy bacillus grows in a medium under aerobic conditions, cytochromes c and a may not be produced (as in the case of *M. lepraemurium*) in which case catalase would need to be produced because if the leprosy bacillus lacks catalase coding DNA, it will not grow under aerobic conditions. Since *M. lepraemurium* had catalase and NAD-peroxidase activity, it grew on 1% Ogawa egg yolk medium. *M. leprae*, however, may be difficult to grow under aerobic conditions since NAD-peroxidase is not present. Katoch, *et al.* ([8, 9]) reported that the leprosy bacillus had no T-catalase, but they did detect some M-catalase which remained after neutralization by an anti-armadillo liver catalase antibody. They reported that the anti-catalase antibody inhibited purified armadillo liver catalase activity, but they did not describe the crude catalase activity of the armadillo liver. Although the cell surface catalase activity of the leprosy bacillus may be degraded by alkali treatment, the inner cell may be left intact. Weak catalase activity would be detected in the cell-free extract of *M. leprae* because of our sensitive catalase determination method.

Wheeler, *et al.* ([26]) detected some glycolysis enzyme activity in *M. leprae*, but we could not detect either glyceraldehyde-3-phosphate dehydrogenase or α-glycerophosphate dehydrogenase in the cell-free extract of *M. leprae*. Wheeler used the Draper method to collect the leprosy bacilli, but that bacterial fraction may have been contaminated with armadillo tissue. Since *M. leprae* lack catalase and NAD-peroxidase activity, *M. leprae* could be cultivated under anaerobic conditions if the glycolysis enzyme system exists in the bacilli. We know of no reports in the literature regarding the anaerobic growth of mycobacteria. In fact, *M. smegmatis* ATCC 23011 could not be grown by the gas pack method under absolute anaerobic conditions.

Leprosy bacilli which do not have the ability to produce ATP under anaerobic conditions may be unable to grow under anaerobic conditions also. The terminal electron transport system of *M. leprae* is summarized in Figure 11. Since the oxidation system of some flavine enzymes produces hydrogen peroxide, perhaps *M. leprae* should be cultivated in low-oxygen tension in order to avoid the formation of excessive hydrogen peroxide. DOPA-oxidase ([24]) may participate in the electron transfer in *M. leprae*, with a large volume of oxygen irreversibly oxidizing DOPA to DOPA-chrome. The most important problem in cultivating

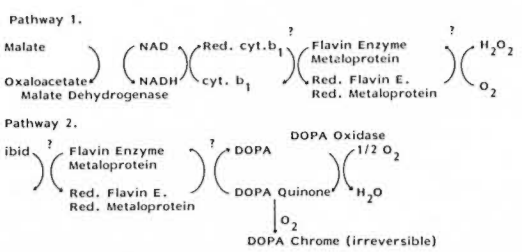


FIG. 11. Terminal electron transport system of *M. leprae*.

*M. leprae*, therefore, may be the interaction with oxygen.


## SUMMARY

Fairly pure leprosy bacilli were easily collected from nude mouse foot pad lepromas by the Ficoll density gradient centrifugation and alkali treatment methods. The yield of bacilli available for biochemical study was 42.6%. The density of *Mycobacterium leprae* was very heterogeneous. The percent of solid bacilli in the light bacilli fraction was 23%; that in the heavy bacilli fraction was 40%. The endogenous respiration activity in the heavy bacilli was greater than that in light bacilli. The average coefficient of respiration in *M. leprae* was 1 $\mu$l $O_2$/mg·hr. In the whole cells of *M. leprae*, a cytochrome $b_1$ absorption peak and its Soret peak were detected at wavelengths of 560 nm and 426 nm, respectively. However, a cytochrome $a_2$-like peak (which was observed in *M. lepraemurium*), and a cyt c and cyt a were not detected. Catalase activity was not found in whole cells, the cell-free extract, or particle fractions of *M. leprae*. Any catalase activity associated with *M. leprae* suspensions is a tissue contaminant. NAD-peroxidase activity was also not detected in the cell-free extract of the leprosy bacillus. These results would indicate that leprosy bacilli cannot degrade hydrogen peroxide.

## RESUMEN

Por centrifugación en gradiente de densidad con Ficoll y tratamiento alcalino se aislaron con facilidad los bacilos de la lepra a partir de lepromas en los cojinetes plantares de ratones desnudos. El rendimiento de bacilos purificados fue del 42.6% de los bacilos originales. La densidad del *Mycobacterium leprae* fue muy heterogénea. El porcentaje de bacilos sólidamente teñidos en la fracción bacilar ligera fue del 23% mientras que en la fracción más densa fue del 40%. La respiración endógena fue mayor en los bacilos pesados que en los ligeros. El coeficiente promedio de respiración del *M. leprae* fue de 1 $\mu$g $O_2$/mg/hr. En los bacilos íntegros se encontraron dos picos de absorción correspondientes al citocromo $b_1$ y a su pico Soret (560 nm y 426 nm respectivamente) pero no se encontraron los picos correspondientes al citocromo $a_2$ (como el observado en *M. lepraemurium*) ni a los citocromos c ó a. No se encontró actividad de catalasa en las células íntegras, en los extractos libres de células, ni en las fracciones particuladas del *M. leprae*. Cualquier actividad de catalasa asociada con suspensiones del *M. leprae* resulta de contaminación tisular. Tampoco hubo actividad de NAD-peroxidasa en los extractos libres de células del bacilo de la lepra. Estos resultados sugieren que el bacilo de la lepra no puede degradar al peroxido de hidrógeno.

## RÉSUMÉ

Des bacilles de la lèpre purifiés ont été récoltés sans difficultés à partir de lépromes du coussinet plantaire de la souris glabre selon la méthode de Ficoll (centrifugation par gradients de densité et par un traitement aux alcalins. On a pu ainsi obtenir un rendement de bacilles purifiés pour études biochimiques, s'élevant à 42,6% de la quantité de bacilles traitée au départ. La densité de *Mycobacterium leprae* était très hétérogène. La proportion de bacilles solides dans la fraction bacillaire légère était de 23%, alors qu'elle était de 40% dans la fraction bacillaire lourde. La respiration endogène était plus intense pour les bacilles de la fraction lourde que pour les bacilles de la fraction légère. Le coefficient moyen de respiration de *M. leprae* s'est élevé à 1 $\mu$l d'oxygène/mg, et par heure. Dans les cellules entières de *M. leprae*, on a pu mettre en évidence un pic d'absorbtion du cytochrome $b_1$ et de son pic de Soret à des longueurs d'onde de 560 nm et de 426 nm respectivement. Par contre, il n'a pas été possible de détecter un pic du type du cytochrome $a_2$ (ainsi qu'on peut l'observer chez *M. lepraemurium*), non plus que des pics pour le cytochrome c et le cytochrome a. Aucune activité catalasique n'a été décelée dans les cellules intactes, dans l'extrait libre de cellule, ou dans les fractions particulées de *M. leprae*. Toute activité en catalase associée à des suspensions de *M. leprae* constitue une contamination tissulaire. Il n'a pas davantage été possible d'identifier une activité en NAD-peroxydase dans l'extrait libre de cellules de bacilles de la lèpre. Ces résultats semblent indiquer que les bacilles de la lèpre ne peuvent pas dégrader le peroxyde d'hydrogène.


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