Adenosine Triphosphate Content in *Mycobacterium leprae*. A Brief Communication[1]

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The bacterial cultivation of *Mycobacterium leprae* is often regarded as the bottleneck that prevents adequate investigations of key problems in leprosy. Major impediments to the cultivation of "obligate intracellular parasites" such as *M. leprae* have been: a) lack of biochemical tools for assessing the energy status of host-grown microbes held under *in vitro* conditions and b) failure to define a cluster of compounds that promote growth of such noncultivable agents.

Scientists at the Goddard Space Flight Center (United States National Aeronautics and Space Administration) selected adenosine triphosphate (ATP) as the primary indicator of extraterrestrial life ([10]) because ATP is unique to bioenergetics and synthesis and because of the specificity ([11]) and exquisite sensitivity of the firefly bioluminescence system of McElroy and associates ([8,9]). In the early 1970s these considerations appealed to us as a means of quantitating the growth potential of *M. leprae* during disease and during experimentation *in vitro*. If ATP cannot be generated, its utilization and degradation rapidly deplete the original supply. Low rates of generating ATP provide energy of maintenance and a steady state; slightly higher levels promote slow growth while maximal production causes rapid growth. Furthermore, during growth at a given rate, the ATP content per cell is constant, and provided the cells are under standard conditions, the ATP per culture or aliquot can measure cell numbers. Thus, increases or decreases in ATP can distinguish between deteriorating populations and those engaged in genuine growth.

By modifying the firefly bioluminescence method of Chappelle and Levin ([1]) and by using chloroform and heat for extracting bacterial ATP ([6]), in 1973 we developed ultrasensitive assays of ATP. These assays provided a tool for assessing the energy status of *Mycobacterium lepraemurium* held *in vitro* ([3]), for quantitating its *in vitro* growth in the Nakamura system ([4,5]), and, further, for improving the Nakamura system to obtain its continuous *in vitro* growth at 38°C ([2]).

In an effort to apply the principles learned from *M. lepraemurium*, determinations of ATP in *M. leprae* were undertaken, and preliminary results are discussed in this communication.

MATERIALS AND METHODS

Liver from an armadillo infected with human-derived *M. leprae* was received from Dr. W. F. Kirchheimer, USPHS Hospital, Carville, Louisiana. Two foot pads from a nude mouse also infected with human-derived *M. leprae* were received from Dr. Tonetaro Ito, Osaka University, Japan. Frozen tissues were thawed at room temperature and homogenized in 4× volumes of $AlbYS_{15}$ (15% bovine serum albumin, Fraction V, and 15% Difco Yeast Supplement B) using a glass homogenizer. The suspensions were centrifuged for 6 min at 200 × *g* and the supernatants used for assays. The microscopic counts were made by the pin head method ([7]). As controls, liver from noninfected armadillos was processed in a similar way, keeping weights and volumes in the same proportions as above. Armadillo tissue suspensions were diluted 400 fold with 0.05 M Tris buffer (Tris [hydroxymethyl] amino methane), pH 7.7, containing 0.01 M $MgSO_4$ while the foot pad bacillary suspension was diluted

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1500 fold in the same buffer. One ml portions from each were centrifuged for 20 min at 3000 × *g* and the sediments suspended in 0.13 ml Tris buffer; 0.03 ml were removed for microscopic counts, and the remaining 0.10 ml was used for ATP extraction by the method of Dhople and Hanks ([6]). The final extract was suspended in 0.4 ml Tris buffer.

For ATP assays, DuPont premixed luciferin-luciferase reagent was used. The lyophilized content from one vial of enzyme-substrate was rehydrated in 4.5 ml Tris buffer (0.05 M, pH 7.7 and containing 0.01 M $MgSO_4$) and incubated overnight at 4°C; 0.3 ml of this was used for each assay with 0.1 ml of ATP extract. An Aminco Chem-Glow Photometer (American Instrument Co., Silver Springs, Maryland, U.S.A.) was used for the ATP determinations.

RESULTS AND DISCUSSION

The suspension prepared from the armadillo liver contained 8×10^9 *M. leprae* per ml (4×10^{10} per gm tissue) while that prepared from the nude mouse foot pad contained 3×10^{10} *M. leprae* per ml (1.5×10^{11} per gm). The morphological index of the *M. leprae* in each suspension was 30–35%.

The ATP content per 1 ml aliquot of diluted suspension was 27.48 pg in the case of *M. leprae* harvested from infected armadillo liver and 24.57 pg in the case of those harvested from the nude mouse foot pad. The suspension from uninoculated armadillo liver, when diluted in the same proportion as above, did not contain any detectable ATP. On correlating the ATP data with microscopic counts, the ATP content per one million *M. leprae* was found to be 1.30 pg and 1.17 pg respectively for organisms harvested from armadillo liver and nude mouse foot pad.

The data clearly suggest that *M. leprae* harvested from either infected armadillo liver or infected nude mouse foot pad contain on the average 1.24 pg ATP per 10^6 cells, which is about 50% of that obtained from *M. lepraemurium* (2.4–2.6 pg). Two points suggest that the ATP found in these *M. leprae* suspensions represent intracellular ATP and do originate from host tissues:

a) In uninoculated armadillo liver, when dilutions were made similar to those made with infected tissue, no detectable ATP was found, indicating that all host-originated ATP is eliminated during dilutions and centrifugations. Thus, all the ATP in infected armadillo liver would seem to be derived from *M. leprae*.

b) The quality and quantity of host material from the two sources, infected armadillo and nude mouse, were totally different. However, the ATP content per aliquot after different dilutions (400 fold and 1500 fold) was almost the same, i.e., 27.5 pg and 24.5 pg. This again suggests that the ATP obtained was intracellular and not host derived.

The findings reported here clearly show that *M. leprae* contain an adequate amount of ATP to allow it to be quantitated by the currently available method. *M. leprae* are usually somewhat larger in size than *M. lepraemurium*. However, *M. lepraemurium* harvested from systemically infected mice contain about 2.4–2.6 pg ATP per million cells with about 90–95% of the cells being solidly stained. In the present studies, the morphological index of *M. leprae* was 30–35%. On this basis, it can be postulated that, unit to unit, the ATP content of intact *M. leprae* is approximately the same as that of intact *M. lepraemurium*.

SUMMARY

Using the firefly bioluminescence method, the ATP content of *M. leprae* harvested from the armadillo and nude mouse was found to be about 1.24 pg per one million organisms. This is about half that present in *M. lepraemurium*.

RESUMEN

Usando un ensayo de bioluminisencia, se encontró que el contenido en ATP del *M. leprae* crecido en el armadillo y en el ratón desnudo, fue de aproximadamente 1.24 pg por millón de organismos. Este valor es casi la mitad del encontrado en el *M. lepraemurium*.

RÉSUMÉ

Grâce à une méthode de bioluminescence utilisant des lucioles, on a pu démontré que le contenu en ATP de *M. leprae* recueilli chez des tatous et chez des souris glabres atteignait environ 1,24 pg par million d'organismes. Ceci est à peu près la moitié de la quantité trouvée chez *M. lepraemurium*.

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