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A Mode of the Growth of *Mycobacterium lepraemurium* in a Cell-free Liquid Medium (38501)

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(Introduced by J. H. Hanks)

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In the previous papers (1–3), it was demonstrated that *M. lepraemurium* quantitatively multiplied in NC-5 medium which is an artificial liquid culture medium. In addition, periodical growth patterns of the bacilli on a slide glass in the medium were already illustrated by light microscopic and scanning electron microscopic observations (4, 5). The present paper describes, in particular, a growth pattern of a single cell of *M. lepraemurium* in a cell-free liquid culture medium.

Materials and Methods. M. lepraemurium. A leproma of the Hawaiian strain (M-55) experimentally developed in the subcutaneous tissues of a C_3H mouse was used. The bacillary suspension was prepared with sterile distilled water. Thereafter, the suspension was partially purified by the treatment with pronase at the final concentration of 0.1%, at 37° for 30 min. The bacilli were collected by centrifugation at 1,000 *g* for 30 min, and resuspended with sterile distilled water.

Cultivation. Two tenths mililliter of the bacillary suspension thus partially purified were inoculated to the NC-5 medium (3), and incubated at 30°.

Electron microscopy. Specimens were collected from the cultures every 2 weeks. After centrifuging the culture tube at 1,000 *g* for 20 min, culture fluid was discarded and the pellet was resuspended with 0.2 ml of distilled water. A droplet of the resuspended bacillary suspension was placed on a copper grid coated by formvar film. One minute later, the film was washed thrice with distilled water, then dried at 37°. The sample was shadowed with chromium and examined by and electron microscope, Nippon Denshi, KK., JEM 7 type at 80 kv of accelerating voltage.

Results. Electron micrographs of a single cell during multiplication were selected from those taken every 2 weeks after cultivation and demonstrated here. In generally speaking, it was noted from the results obtained that the multiplication of bacilli was perfected as the following course; elongation, septum formation, and dividing, with some exceptions such as budding and dividing without elongation. Figure 2 shows an elongated bacterial cell which is about 2.5 times as long as that of the original cell (Fig. 1). This finding was mostly observed at the first 2 weeks of cultivation. Next, the septum formation in the elongated cell is clearly shown in Fig. 3. Finally, Fig. 4 shows the typical pattern of a single cell just after completion of dividing. These findings are ordinary growth patterns which are commonly observed in bacterial multiplications. Besides these findings, a budding pattern shown in Fig. 5, was frequently observed. In most cases, a budding took place at the lateral side of the cell, but in a few cases at the terminal

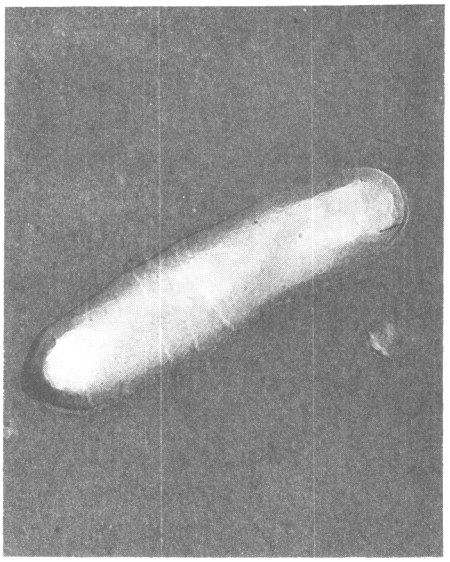

FIG. 1. Electron micrograph of *M. lepraemurium* at "0" time of cultivation (Magnification × 25,600).

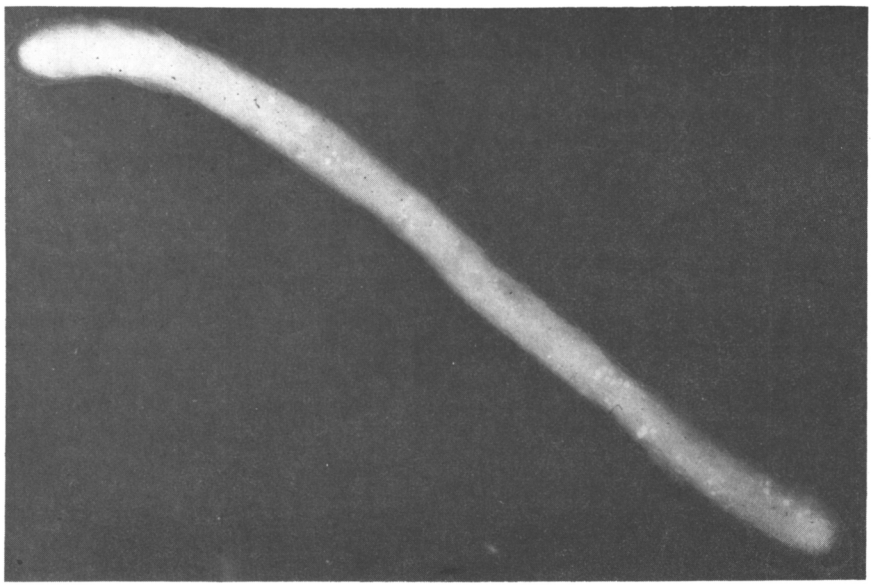

FIG. 2. Elongated cell of *M. lepraemurium* (Magnification × 16,000).

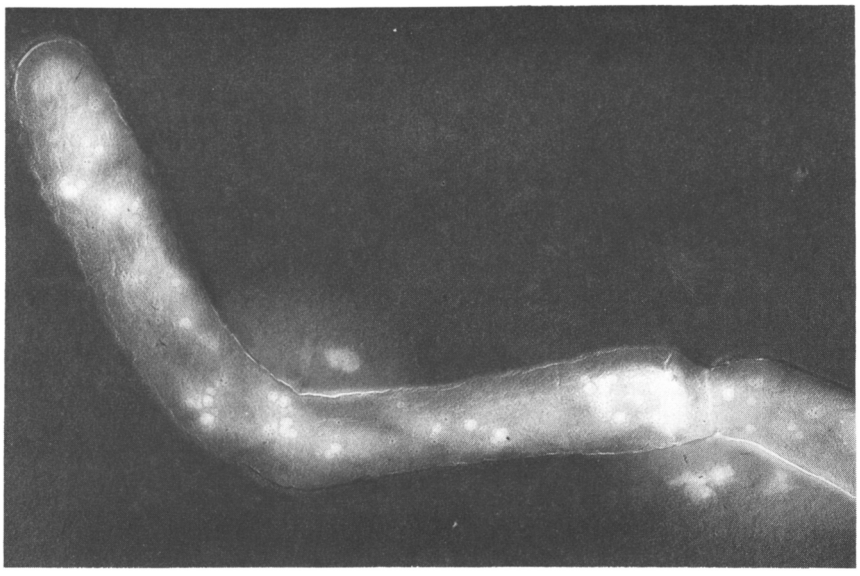

FIG. 3. Septum formation (Magnification × 30,000).

part of the cell. Furthermore, Fig. 6 as an exceptional pattern, is shown. This micrograph illustrates a pattern of dividing without elongation of the cell which had presumably budded.

In addition, an atypical form of the cell is noted in Fig. 7, in which a swelling granule is recognized at subterminal position of the cell. This might be one phase during multiplication or life cycle of the bacillus.

Discussion. The multiplication system of *M. lepraemurium* in NC-5 medium (3) was reliably confirmed by Dhople and Hanks (6). In this paper, growth patterns, especially each stage of the multiplication from elongation to division were demonstrated by using this cultivation system. The results obtained indicate that *M. lepraemurium* also multiplies *in vitro* in the ordinary course that is recognized in a common bacterial cell; that is,

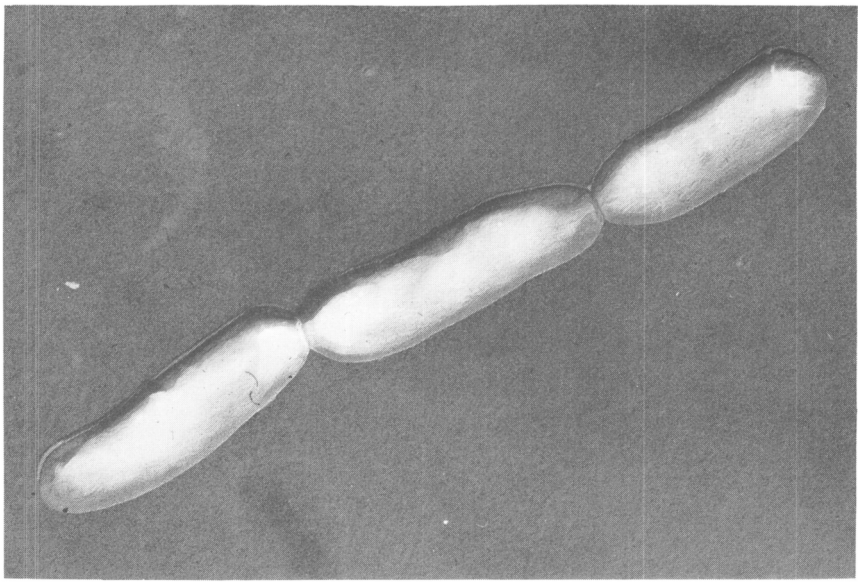

FIG. 4. Cell division (Magnification × 23,000).

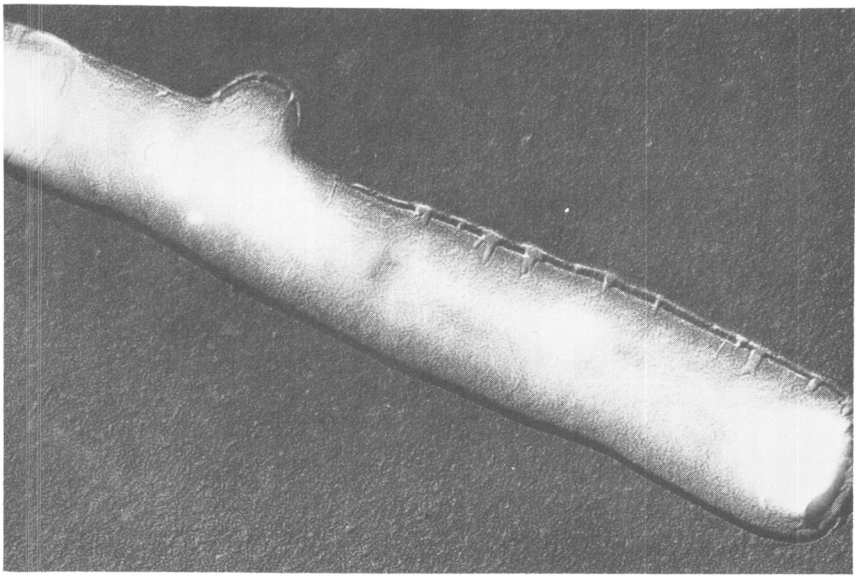

FIG. 5. Lateral budding (Magnification × 34,000).

elongation, septum formation and dividing. In addition, budding patterns were frequently noted. Therefore, it is no doubt that *M. lepraemurium* belongs to *Order Actinomycetales*. It is well known, in general, a cell of Mycobacterium is divided by binary fission. However, in *M. lepraemurium*, the lateral budding which may be a beginning of branching and be frequently recognized in the case of nocardia is sometimes seen. It is considered to be specific growth pattern of *M. lepraemurium*. Furthermore, terminal swellings were seen in the later stages. These findings could also be observed in the growth of nocardia and atypical mycobacteria, *M. marianum* (7). Therefore, it could be proposed that *M. lepraemurium* should belong to a group of atypical mycobacterium. There is no evidence, in this paper, that the septum is consistently formed in an elongated cell. In

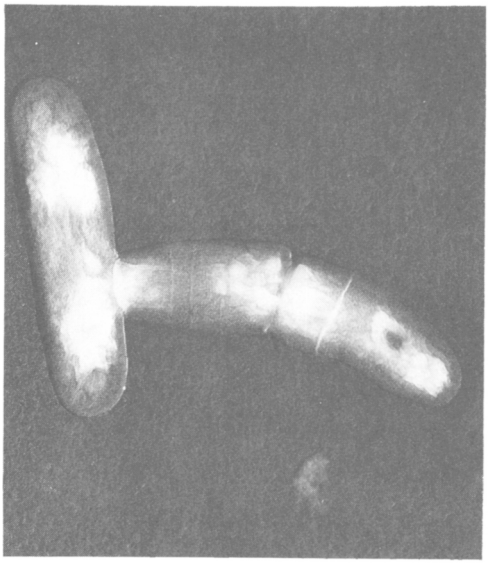

FIG. 6. Division without elongation after budding (Magnification × 25,000).

the future, the ultrathin section method should be employed in order to confirm the septum formation, and to study the inside ultrastructural changes in bacterial cell during multiplication.

Summary. The first report regarding *in vitro* growth patterns of *M. lepraemurium* was presented. Evidence is indicated that a growth mode of a single cell of *M. lepraemurium in vitro* is similar to that of culturable mycobacterium, especially atypical mycobacterium. This finding has added one property to characteristics of *M. lepraemurium* which have been already known.

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1. Nakamura, M., J. Gen. Microbiol. **73,** 193 (1972).
2. Nakamura, M., Proc. Jap. Acad. **49,** 42 (1973).
3. Nakamura, M., J. Gen. Microbiol. **83,** (1974) in press.
4. Yoshii, Z., and Nakamura, M., Proc. Jap. Acad. **49,** 47 (1973).
5. Yoshii, Z., and Nakamura, M., J. Gen. Microbiol. **83,** (1974) in press.
6. Dhople, A. M., and Hanks, J. H., Leprosy Sci. Memo. L-531/1 (1974).
7. Penso, G., "Atlas of Electron Microscopy," Elsevier Publishing Co. (1964).

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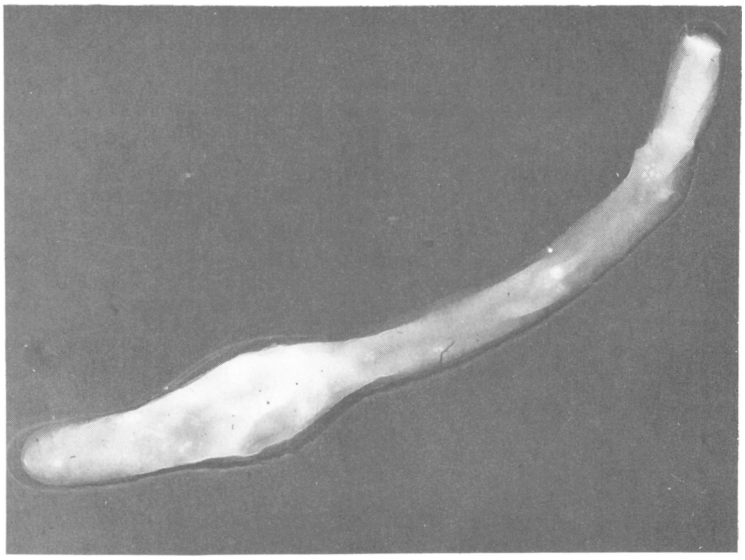

FIG. 7. Terminal swelling (Magnification × 18,000).