Approximately 2 to 3 weeks of continuous cultivation were necessary before cells became persistently infected with rickettsiae. Although the detachment of cells from the glass surface became prominent during the third week of culture, no cytopathology specific to rickettsial multiplication was observable. Intracytoplasmic localization of the rickettsiae at the 12th transfer of infected FL cells is shown in Fig. 1. Dividing cells in late telophase sometimes showed rickettsiae in both daughter cells; or, only one cell might be involved, the other remaining free from infection. Therefore, this kind of cell division may play a role in the maintenance of the carrier state, together with cell-to-cell infections. Detailed studies of the mechanism of establishment of the carrier state reported here are under way.

In summary, an FL cell strain carrying *R. sennetsu* (s. *todai*) was established by infecting the cells in vitro and subculturing them once a month.

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SITES OF ADENOSINE TRIPHOSPHATASE ACTIVITY IN BACTERIA

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The important role of adenosine triphosphatase in permeability and ion transport was suggested by Whittam (Nature **191**:603, 1961). In higher cells, adenosine triphosphatase activity is predominantly membrane-bound, being located in cell membranes and cristae mitochondriales. Adenosine triphosphatase activity has also been isolated from contractile proteins of dividing cells (*General Cytology*, 3rd ed., W. B. Saunders Co., Philadelphia, 1960). In bacteria, this activity has been demonstrated in particulate fractions of the cytoplasmic membrane, in the cell wall, and in cytoplasmic particles from different species (Hughes, J. Gen. Microbiol. **29**:39, 1962). Georgi et al. (J. Bacteriol. **70**:716, 1955) found adenosine triphosphatase activity in dense granules located in the red layer of fractionated bacteria. Since these granules were membrane-bound, a physical separation of the two structures, granules and adhering membranes, could not easily be achieved, to show whether the enzyme is membrane- or granule-bound, or both (Marr, p. 460. *In* I. C. Gunsalus and R. Y. Stanier [ed.], *The Bacteria*, vol. 1, Academic Press, Inc., 1960). There have been no attempts to apply cytochemical methods, in connection with electron microscopy, for localizing the sites of adenosine triphosphatase activity in bacterial cells.

Three species, *Escherichia coli B*, *Bacillus cereus*, and *Myxococcus xanthus FB* (Voelz and Dworkin, J. Bacteriol. **84**:943, 1962), were chosen for these studies. Cultures of all three species were grown in a liquid medium containing 2% Casitone, 0.1% $MgSO_4$, and 0.01 M K_2HPO_4-KH_2PO_4 (pH 7.2) by shaking at room temperature. Cells were harvested at about the end of their logarithmic phase, washed with phosphate buffer, and fixed in 1% glutaraldehyde buffered with phosphate-saline (Dulbecco and Vogt, J. Exptl. Med. **99**:167, 1954) at pH 7.2 for 10 min at room temperature. Studies on the time kinetics of glutaraldehyde fixation have shown that an exposure of cells of the species investigated to the fixative for up to 30 min does not affect the adenosine triphosphatase activity (Voelz, *unpublished data*). Fixed cells were washed five times in distilled water and suspended in the adenosine triphosphate (ATP) medium of Otero-Vilardebo et al. (J. Cell Biol. **19**:647, 1963). Incubation was for 20 min at room temperature. After incubation, the cells were washed three

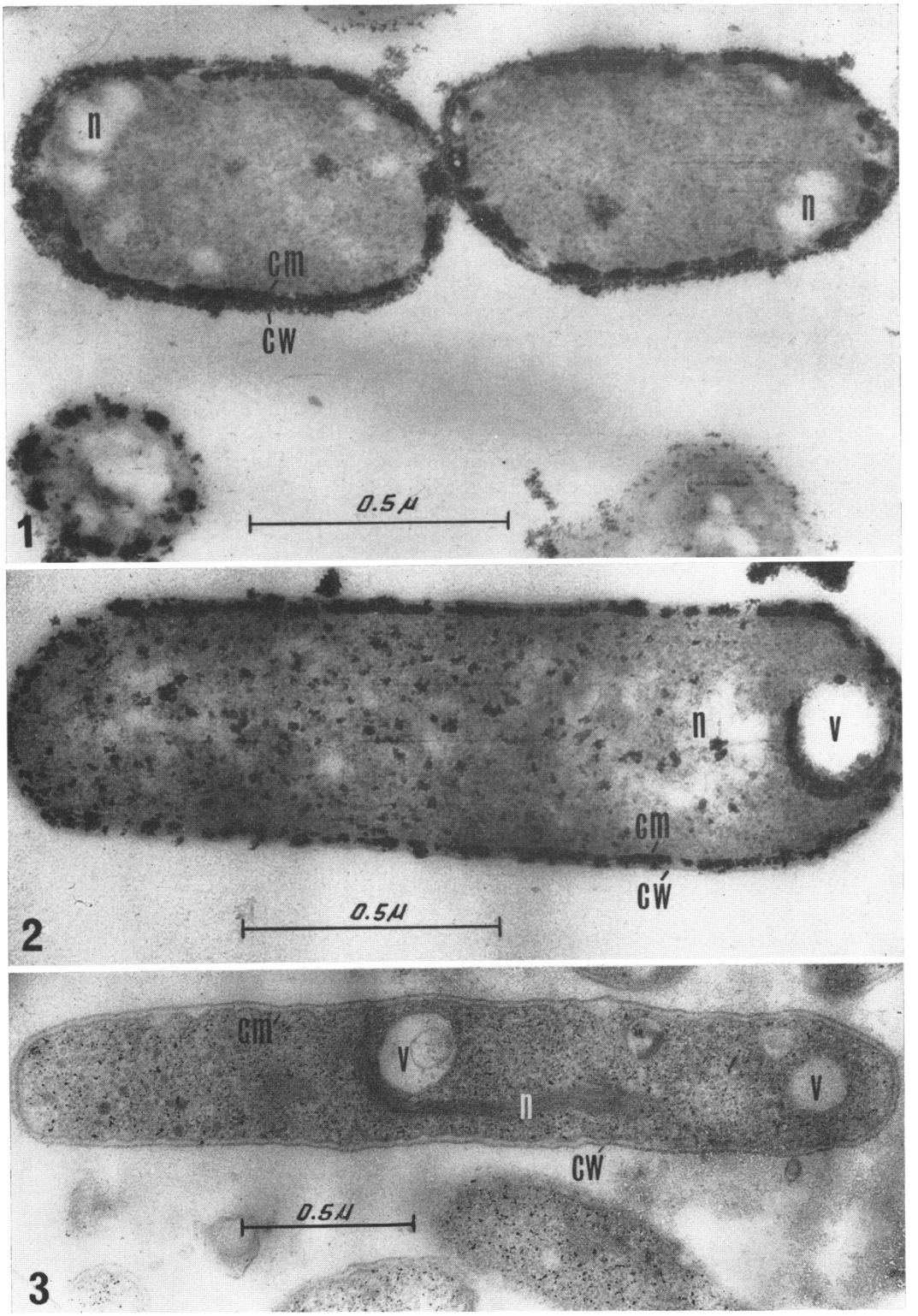

FIG. 1. *Unstained section of Escherichia coli cell showing adenosine triphosphatase activity at the cytoplasmic membrane (CM) and cell wall (CW); n, nuclear material. The nuclear regions were identified by a control stain of serial sections with uranyl acetate.*

FIG. 2. *Unstained section through a cell of Bacillus cereus with adenosine triphosphatase activity at the cytoplasmic membrane, around the vacuole, in the nuclear region and in the cytoplasm; v, vacuole.*

FIG. 3. *Unstained section of a cell of Myxococcus xanthus. Adenosine triphosphatase activity occurs in the cytoplasm only (dense lead-phosphate deposits).*

times in distilled water and treated with 1% OsO_4 for 5 min. Dehydration and embedding were according to the procedure described previously by Voelz and Dworkin. The photographs in Fig. 1 to 3 are sections through cells representative of the cell populations of each of the species.

Adenosine triphosphatase activity was present in all three species investigated, as demonstrated by lead deposits in the cell. These deposits presumably result from the enzymatic cleavage of ATP and the subsequent precipitation of orthophosphate by $Pb(NO_3)_2$, one of the components of the medium. In controls minus ATP, none of the three species tested had lead phosphate deposits. The adenosine triphosphatase reaction is specific, in that orthophosphate is not liberated from a wide spectrum of organic phosphorus compounds, including adenosine monophosphate (*see* Hughes). Electron micrographs demonstrate that the sites of adenosine triphosphatase activity vary among the species tested. In *E. coli* (Fig. 1) adenosine triphosphatase activity was found predominantly in the cytoplasmic membrane and cell wall. In *B. cereus* the enzyme activity was located in the cytoplasmic membrane, cytoplasm, and the nuclear region (Fig. 2). Enzyme activity was also present around the vacuole. No activity was found in the cell wall of *B. cereus*. Adenosine triphosphatase activity in *M. xanthus* (Fig. 3) was found only in the cytoplasm. It is uncertain that the three species were harvested and fixed at the same stage of their particular growth cycle. Enzyme activity or the supply of ATP can possibly vary in certain areas within the cell at different stages of growth. Since adenosine triphosphatase has been isolated from contractile proteins of dividing higher cells, and since a change has been observed in the site of adenosine triphosphatase activity in bone-marrow cells of mice, depending on the stage of differentiation or mitotic cycle (Voelz, *unpublished data*), it is conceivable that adenosine triphosphatase can occur in the cytoplasm of bacterial cells, in the cell wall, or in the cytoplasmic membrane, depending upon their stage of development. This could explain the diversity of adenosine triphosphatase sites found in different bacterial species. Further studies will be necessary to determine whether physiological conditions during growth and morphogenesis influence the sites or activity of this enzyme.

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IMPROVED TECHNIQUE FOR THE PREPARATION OF STREPTOCOCCAL CELL WALLS

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An efficient means for the mechanical disruption of bacterial cells in a Mickle disintegrator with subsequent separation of the cell walls from the cytoplasmic contents by differential centrifugation was introduced by Salton and Horne (Biochim. Biophys. Acta **7**:177, 1951). In this procedure, a suspension of bacteria is vigorously shaken with glass beads; although excellent cell-wall preparations devoid of cellular material can be achieved, considerable time is required to process large batches of bacteria. For this reason other disruption equipment has been employed for batch work, such as the Waring Blendor and the Virtis homogenizer. One of the major disadvantages of these instruments is that the glass beads abrade the metal blades, releasing fine metal particles which cannot be separated from the cell walls with subsequent centrifugation. This technical report describes the disintegration of hemolytic streptococci with a Braun homogenizer (Bronwill Scientific, Rochester, N.Y.). The use of this instrument has proven particularly advantageous, because large batches of streptococci can be efficiently and effectively disrupted in a short time and the isolated cell walls are devoid of metallic particles. The data indicate

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