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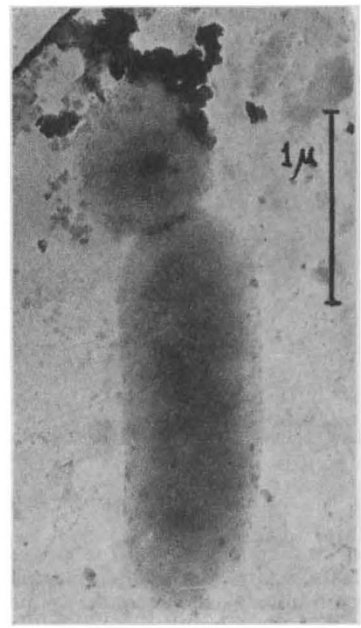
Sectioning of the Bacterial Cell for the Electron Microscope

The introduction of a technique for cutting ultra-thin sections of biological tissue with the conventional microtome[1] has also made feasible the sectioning of isolated cells for the electron microscope. Bacterial cells were chosen as a first example because of convenient size, ease of handling in large numbers, and because of the intrinsic importance of any new approach to the problems of bacterial morphology.

B. megatherium was grown on nutrient agar, and when the culture was approximately twenty-four hours old, quantities of cells were transferred with a wire loop to a 5 per cent formalin solution in a centrifuge tube. After one hour of fixation the cells were centrifuged for five minutes at 20,000 r.p.m. and the formalin then poured off. Dehydration with a graded series of alcohols was then carried out, using the centrifuge to keep the bacteria together in the transition from one solution to another. Impregnation with Mallinckrodt 'Parlodion' followed, starting with 3 per cent 'Parlodion' in equal parts of ether-alcohol and ending in a 12 per cent solution. Transition from 6 to 12 per cent 'Parlodion' was accomplished by evaporation of the solvent. After one day in 12 per cent 'Parlodion', chloroform was used as a hardening agent, after which the hardened block was broken out of the centrifuge tube. Impregnation of the block with paraffin (m.p. 65°) followed, after going through a carbol-xylol bath to remove the last traces of water. The doubly embedded block was finally mounted on a plastic pillar which could be clamped in the microtome.

One-tenth micron sections were cut as described in ref. 1, and were flattened on a microscope slide, using needles under a binocular dissecting microscope. The paraffin was removed with benzol, after which the slide was dipped vertically into a beaker of dilute (0·2 per cent) collodion in amyl acetate. This replaced the original collodion with a thinner and more homogeneous film, which was then floated off on a clean water surface. Microscope specimen screens (200 mesh) were placed on the sections and the entire film inverted and mounted on another slide. After drying, the specimens were ready for examination in the electron microscope.



Transverse and longitudinal sections of *B. megatherium* approximately 0·1 microns thick. × 25,000; 50 kV. Note similar density and detail in the two cells

Since the cells were oriented at random in the block, the sections might be expected to show cells cut at various angles. The accompanying photograph shows two cells which were nearly at right angles in the block, giving rise to a longitudinal and a transverse cut in the same field of view. Sufficient internal detail is visible in both cells to make it clear that the sections are adequately thin for effective use with a 50-kV. electron microscope. Much further work will, of course, be necessary to establish the significance of the internal detail observed in an experiment of this type. It is the purpose of this note merely to describe a new technique, which may profitably be used in the study of bacterial morphology and the relationships of bacteria and bacterial viruses.

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A Method of Sectioning Bacteria *in situ* for Electron-microscopical and Cytochemical Investigations

A method has been developed for preparing sections of bacterial cells for observation with the electron microscope. An impression preparation of a young growing culture is made on a polished glass surface and fixed with osmic acid. On to this a beryllium film is evaporated by means of Hast's method[1]. The beryllium film is then stripped from the glass surface and frequently carries with it a section through the bacterial cells with their relationships undisturbed. During the evaporation of the