LETTERS TO THE EDITORS

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Electron Microscopy of Bacteria

BACTERIA have usually been prepared for observation in the electron microscope by growth in liquid media, a drop of the suspension being allowed to dry on a collodion-covered specimen grid. Smiles[1] has employed an adaptation of the impression technique used in optical microscopy for obtaining material from a surface culture: a glass slide pressed on to the agar removes a film of bacteria, which is allowed to dry partially, and then removed by stripping with a collodion film. Hillier and Baker[2] have described a more direct method, in which the collodion film is formed on the agar surface and then floated off in water. Unfortunately, most bacteria, however mounted, prove to be too opaque to an electron beam of the energy usually employed (50–60 kV.) to show appreciable internal structure.

In studying the morphology of avian tubercle bacilli, which, in certain stages of development, we find to be less dense to electrons than most bacteria, we found it impossible to strip them from the Löwenstein and blood-agar surfaces on which they are normally grown. Two methods have been devised by which intact films can consistently be obtained. In the first, a thin coating of glycerine-agar is spread on the surface of the Löwenstein medium and the bacterial inoculum placed on this. After sufficient growth has developed, a 1 per cent solution of collodion is flowed over the surface, allowed to dry, and removed by immersion in water. Alternatively, a square of the Löwenstein medium, at the required stage of growth, is inverted and the surface pressed firmly for a few seconds on to a glycerine agar surface, so that the culture is transferred from the one to the other. It may afterwards be removed from the latter as before by stripping on a collodion film. In either case the specimen grids are then placed on the stripped film, and both removed and mounted in the usual way. The resulting electron micrographs show a richness and clarity of internal structure not otherwise obtainable (Figs. 1–4). Before spreading the collodion film on the culture, it is fixed by exposure for 5 min. to the vapour of 2 per cent osmic acid. As a further precaution against infection, a 5 per cent solution of formalin is employed for floating off the collodion films, which are then washed in distilled water before mounting. Fixation in formalin vapour proved unsatisfactory in that the films could not afterwards be stripped at all.

An interpretation of the results obtained at different stages in the life of a culture will be published elsewhere.

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[1] Smiles (demonstrated to the third Electron Microscope Conference, National Institute for Medical Research, London, June 1945).
[2] Hillier and Baker, *J. Bact.*, **52**, 411 (1946).

A New Isotope of Tungsten

A SEARCH for tungsten radio-isotopes was made using a 5-mil tantalum foil which had received 80 micro-ampere-hours bombardment with approximately 20 MeV. deuterons from the 60-in. Crocker Laboratory cyclotron. The foil was dissolved in nitric acid containing five per cent of hydrofluoric acid. A few milligrams of tungsten carrier was added, and after removal of hydrofluoric acid by fuming with sulphuric acid, the tantalum was precipitated by sodium hydroxide and the precipitate digested with hot alkali. Tungsten was precipitated from the alkaline solution by boiling with excess nitric acid. The procedure was repeated until the tantalum-scavenging carrier precipitates were inactive.

The tungsten fraction contained a previously unreported, single radioactivity of half-life 140 ± 2 days, emitting electrons, X-rays and gamma-rays. The radiations were studied using aluminium, beryllium and lead absorbers, measurements being made with an 'end-on' type Geiger–Müller counter with a 2·7 mgm./cm.^{2} mica window, and filled with 10 cm. argon and 0·5 cm. alcohol. The radiations had the following characteristics.

(1) Electrons of range ~ 11 mgm./cm.^{2}, corresponding to an energy of about 90 keV. The range was determined by resolution of the aluminium absorption curve (Fig. 1) and by direct measurement using thin beryllium absorbers. The absorption of the soft X-ray background was measured in aluminium after removal of electrons by a 14 mgm./cm.^{2} beryllium absorber.

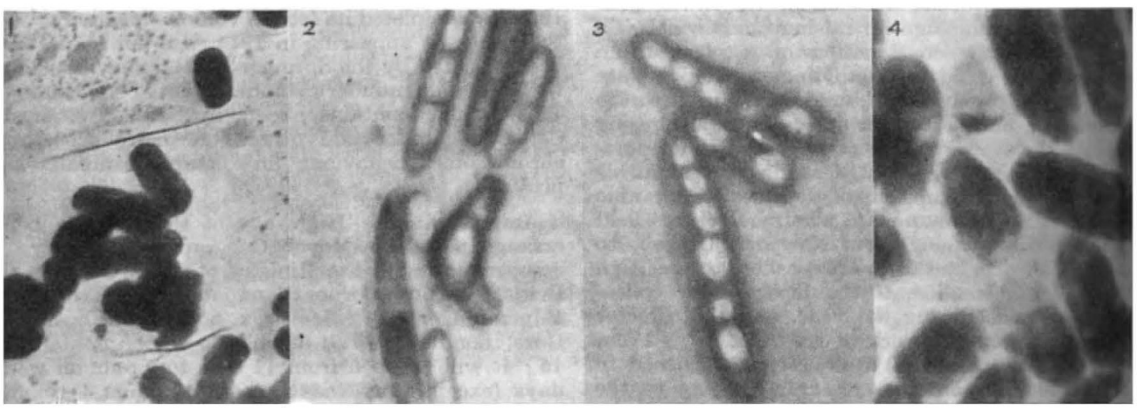

Fig. 1. INOCULUM OF AVIAN TUBERCLE BACILLI, FROM A CULTURE FOUR WEEKS OLD
Figs. 2 and 3. INTERMEDIATE FORMS AFTER 24 HOURS AND THREE DAYS INCUBATION ON LOEWENSTEIN MEDIUM
Fig. 4. FORMATION OF DAUGHTER RODS AFTER FOUR DAYS. × 15,000