THE MOUNTING OF BACTERIA FOR ELECTRON MICROSCOPE EXAMINATION

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The use of the electron microscope in bacteriology has yielded much new information regarding the structure of the bacterial cell (Mudd, 1944; Mudd and

FIG. 1. AN ELECTRON MICROGRAPH OF ESCHERICHIA COLI MOUNTED ON COLLODION FROM A DISTILLED WATER SUSPENSION AND HEAVILY SHADOWED WITH GOLD TO SHOW THE WRINKLED SURFACE

Anderson, 1944). On the other hand, the possibility has always been recognized that certain relationships might be altered by artifacts introduced by the treat-

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ment which the organisms undergo in the preparation of the specimens for the electron microscope. Recent work in this laboratory has provided some information regarding the existence and nature of such artifacts and has led to a new method of preparation that avoids many of the difficulties in the case of some organisms that can be cultured on solid media.

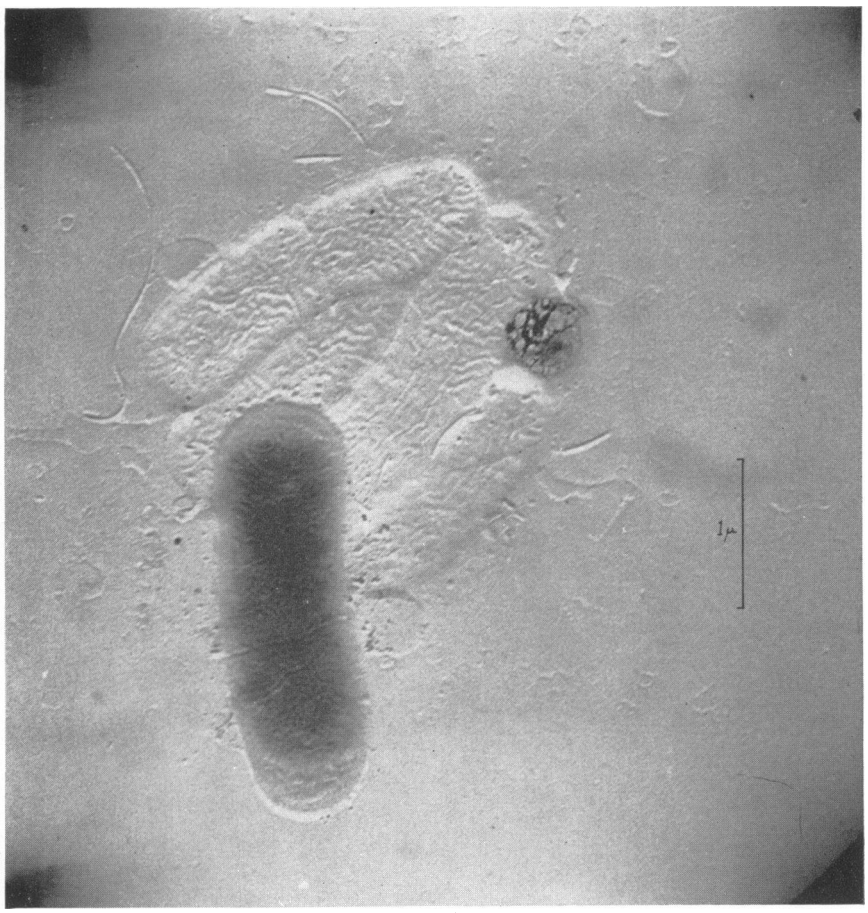

FIG. 2. AN ELECTRON MICROGRAPH OF A GOLD-SHADOWED REPLICA OF SERRATIA MARCESCENS

The bacteria were mounted on a glass slide from a distilled water suspension. The replica was made of this mount and subsequently shadowed. One cell in the field shown adhered to the collodion.

The ordinary method of mounting bacteria for examination in the electron microscope involves suspending the organisms in distilled water, placing a small drop of the suspension on a specimen screen provided with a supporting membrane, and allowing the water to evaporate. There are several points in this procedure at which artifacts may be produced, including the initial disturbance of the bacteria on removal from the culture, the effects of the distilled

water, and the subsequent desiccation. Placing the mounted specimen in the vacuum of the electron microscope and subjecting it to intense electron bombardment may also introduce artifacts.

Relatively little work has been done on the determination of the exact nature and extent of these artifacts. From an investigation of the effects of distilled

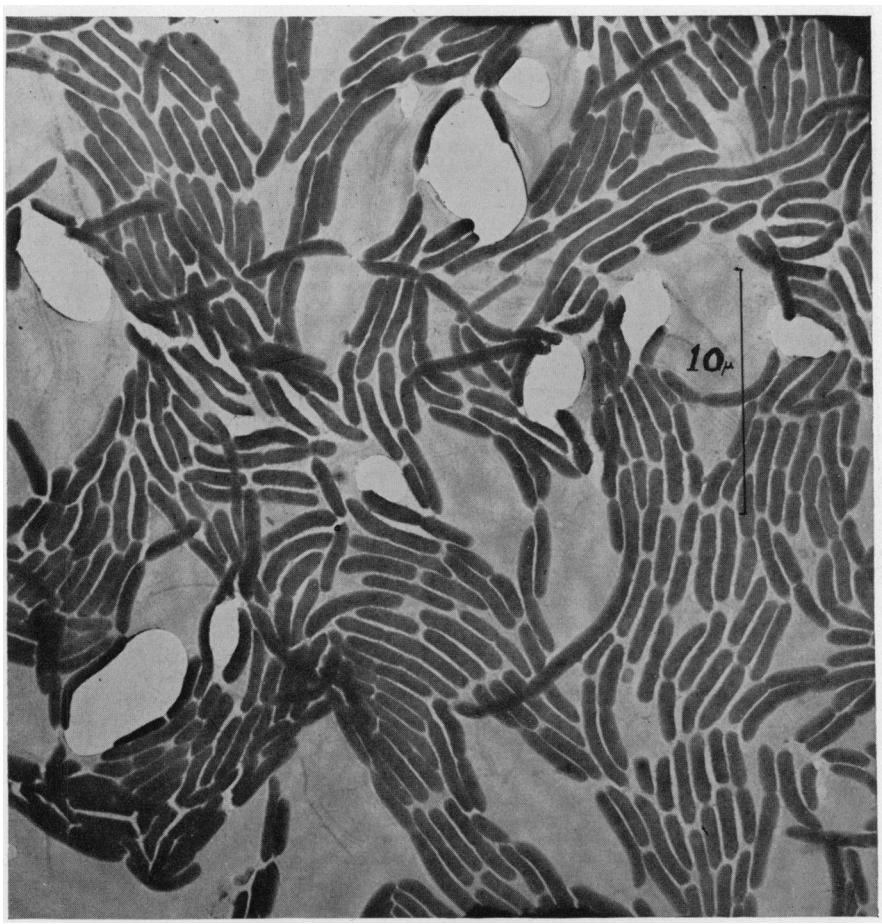

FIG. 3. A LOW MAGNIFICATION ELECTRON MICROGRAPH OF SERRATIA MARCESCENS PREPARED ACCORDING TO THE METHOD DESCRIBED

This specimen was taken from a 5-hour agar plate culture

water on *Bacillus subtilis* and *Escherichia coli* it was concluded that, although there is a steady disintegration of the cells in suspension, the first few minutes do not appreciably affect the electron microscope images (Hillier and Kurkjian, 1944). This work did not exclude the possibility that there is a sudden change of morphology when the suspension is made. A light microscopic investigation of the effect of vacuum on bacterial films gave negative results within the resolu-

tion limits of the instrument. High magnification observations in the electron microscope have indicated that, except at extremely high electron intensities, there is no change in morphology as bacteria are brought into the electron beam.

In the present work a further investigation of the artifacts in electron micrographs of bacteria has been conducted using various modifications and combina-

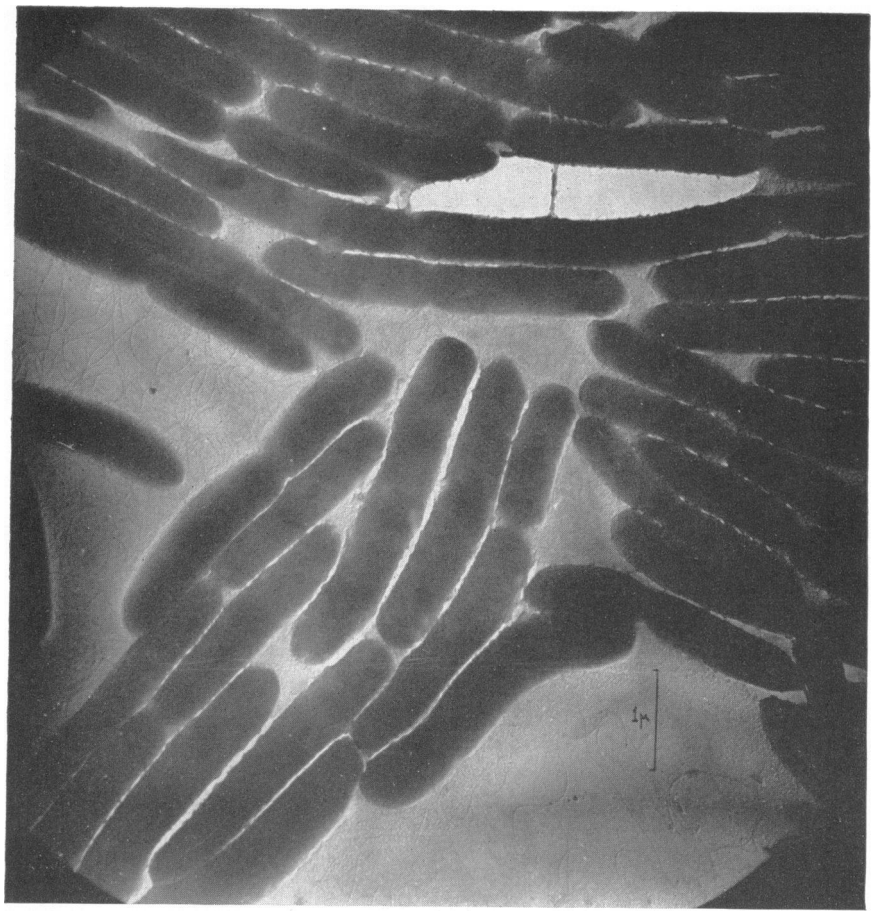

FIG. 4. HIGHER MAGNIFICATION ELECTRON MICROGRAPH OF A SIMILAR PREPARATION SHOWING SOME INTRACELLULAR GRANULES AND A SLIGHT EVIDENCE OF SHRINKAGE

tions of the replica technique of Schaefer and Harker (1942) and the shadowing technique of Williams and Wyckoff (1946). It was observed first that many types of bacteria presented a characteristic wrinkled and flattened appearance when mounted in the conventional way and subsequently heavily shadowed with gold (figure 1). It was then found that gold-shadowed replicas of bacterial films from distilled water presented the same appearance (figure 2). Now the cells of which replicas were obtained had *not* been in a vacuum, since, in this

technique, a collodion replica was made of a dried film on a microscope slide *before* shadowing. It can be concluded, therefore, that the wrinkling and flattening is a result of the initial desiccation and that introduction into the vacuum of the microscope introduced little, if any, further change. Replicas of dried bacteria which had been dispersed initially in a fixing agent showed the cells to be smooth and rounded.

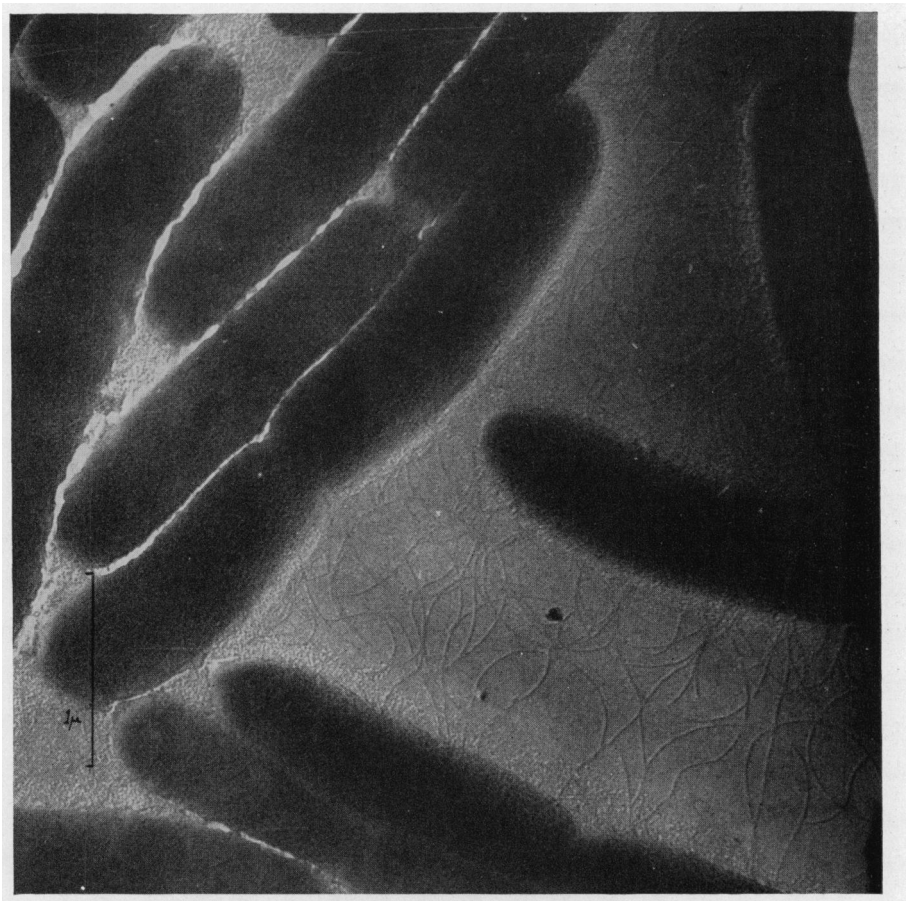

FIG. 5. AN ENLARGEMENT OF PART OF FIGURE 4 SHOWING THE GROWTH OF THE FLAGELLA ON THE SURFACE OF THE AGAR AND A FINELY DIVIDED MATERIAL SURROUNDING THE BACTERIAL CELLS

These observations led to an attempt to obtain a replica of a growing agar plate culture. The technique which was tried consisted simply of flowing a 1 per cent solution of collodion in amyl acetate over a small and young colony, allowing it to evaporate to dryness, floating the collodion film off on a water surface, and shadowing the replica surface with gold after mounting and drying. The technique produced the unexpected results shown in figures 3 to 5. Instead of obtaining a replica we found that a surface layer of the culture was removed

intact by the collodion and that the subsequent handling produced little, if any, change in the appearance of the preparation.

It now appears that, for the study of some bacteria which can be grown on agar-like solid media, this technique has advantages over the conventional one. The most obvious advantage comes from the possibility of studying the bacteria in the environment in which they were grown. Flagella and bacterial excretions which are usually distorted or lost in the older method of preparation can now be studied without disturbing their exact relationship to the bacteria in the growing colony. In fact, the appearance of the flagella in figure 5 suggests to the authors that they may perform functions other than as organs of locomotion. Since the physical relationship of the cells in the growing colony is preserved, it should now be possible to make an accurate correlation between changes in morphology and the processes of cell division. Although the method does not inherently eliminate the possibility of artifacts due to contact between the cells and distilled water, none of the characteristic artifacts have been observed—a fact which may possibly be due to a fixing action of the collodion solution.

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