LETTERS TO THE EDITORS

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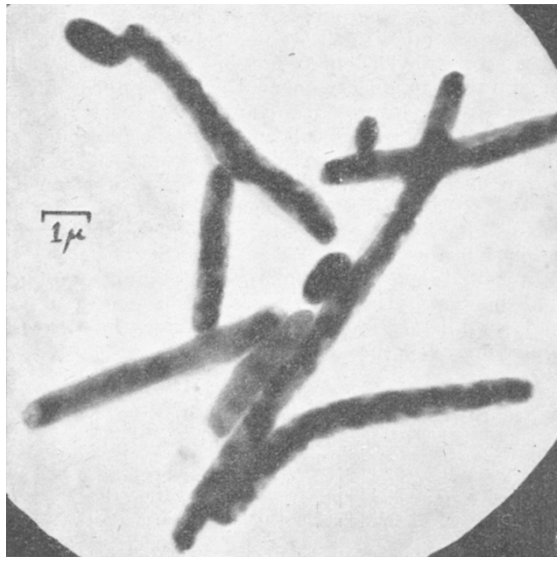
A New Procedure for Bacterial Viability Counts and its Biophysical Applications

A BASIC problem in microbiology is that of determining the proportions of live and dead organisms in a bacterial culture. The standard procedure is to spread a known volume of diluted culture over a solid nutrient medium and then, after incubation, to count the colonies which develop. On the assumption that each colony came from one bacterium, the number of viable organisms per millilitre of original culture can be calculated (but not the percentage viability).

We have found useful a completely different method which consists simply of counting in an electron microscope the live and dead organisms in a random sample of any culture. Live and dead bacteria cannot normally be distinguished from each other. However, if the culture is incubated for a few hours under conditions which permit cell-growth but inhibit cell-division, the live organisms will grow to many times the length of their dead companions and the two kinds can readily be differentiated and counted (see photomicrograph).

A convenient agent for inhibiting division but permitting growth is urea at a concentration of 3 per cent in nutrient agar[1]. The bacteria are grown on a collodion film supported on a stainless-steel electron-microscope specimen grid in a manner similar to that described by Brieger and Cosslett[2]. The grids are placed film downwards on a Petri dish of nutrient agar containing 3 per cent urea. A drop of bacterial culture is then placed on the centre of each grid and the whole incubated at 37° C. for 3 hr. (6 hr. for irradiated bacteria, to allow for the longer lag phase). The grids are then fixed in vapours of osmium tetroxide or formalin, cleaned by floating on distilled water, and finally dried and examined. The live bacteria in the drop will have formed long filaments, whereas the dead ones remain unchanged. Provided the distribution of bacteria on the grid is suitable, a count of the live and dead cells can then be made. Test-counts on suspensions containing known proportions of live and heat-killed bacteria gave answers correct to within the expected sampling error, which is small if several hundred cells are counted. Examination in an electron microscope is probably essential for making accurate counts—especially for distinguishing very short rods from particles of medium or detritus, and for including lysed cells.



A typical field of paracolon bacilli after 2½ hr. growth at 37° C. on division-inhibiting medium (3 per cent urea-agar), showing seven live and three dead organisms

The 'urea method' is often less time-consuming and troublesome than the standard method. Its main disadvantage is that it cannot be applied to cocci, which do not respond to urea by developing into giant forms, or to cultures of organisms which always contain filamentous cells in spite of being cultured under conditions favouring short forms. But we have used it successfully with strains of *Bacterium*, a paracolon bacillus, *Chromobacterium*, and *Pseudomonas*. With suitable strains, counting by the urea method has several important advantages over the standard procedure: (*a*) it is not affected by bacterial clumping, which can entirely vitiate the standard procedure[3]; (*b*) it gives percentage viability, instead of only a total live count per unit volume; (*c*) the dead cells can be identified and examined; (*d*) only a very small sample is required.

The method has proved very useful in studying the resistance of bacteria to drying in fine droplets (as happens so often in Nature), and has also thrown new light on the effects of radiations on bacterial cells. In the latter case the counts may differ widely from those made by the standard procedure, since the urea method measures primarily radiation effects on the systems responsible for cell growth. It presumably does not detect effects on the systems responsible for cell division and heredity, which do, however, affect counts made by the standard procedure. Clearly, in most experiments on bacterial killing, fuller information can be obtained by using the two methods in parallel.

Further details of this and other work based on the urea method will be published elsewhere. One of us (R. C. V.) is indebted to the Agricultural Research Council for support during this work.

R. C. VALENTINE
J. R. G. BRADFIELD

Cavendish Laboratory,
Cambridge.
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[1] Wilson, W. J., *J. Path. Bact.*, **11**, 394 (1906).
[2] Brieger, E. M., and Cosslett, V. E., *Nature*, **164**, 352 (1949).
[3] Lea, D. E., "Actions of Radiations on Living Cells", 317 (Cambridge, 1946).

Applications of Electrophoresis in studying New Antibiotics

THE application of paper electrophoresis to the study of new antibiotics[1] has been in use at these laboratories for some time. Our technique consists of applying spots of culture fluid, or a concentrate of it, to paper strips soaked in buffer, and subjecting the strips to electrophoresis by the technique of Kunkel and Tiselius[2]. Spots of chloromycetin are applied to the paper on each side of the spot of unknown antibiotic as a control for electro-osmosis. After migration, the position of the antibiotic is revealed by the microbiological technique as usual