Conversely, a reduction of nitrate was not observed by those organisms characterized by a lack of this ability. In no instance was a positive test observed that could be attributed to nitrite carry-over in the large inoculum employed.

An aseptic technique was not found to be essential. Accordingly, the test was simplified and shortened by employing tubes that were clean, but not sterile. Cotton plugs were not used. In no instance did a small number of contaminants multiply enough to affect the test results.

A SIMPLE METHOD FOR THE AUTOMATIC SEPARATION OF SMOOTH BACTERIAL TYPES FROM MIXED POPULATIONS

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For the production of vaccines, as well as for many experimental studies on pathogenic bacteria, it is desirable to have a simple method that assures the use of inocula consisting of smooth types only. Ordinarily one may take advantage of the frequently linked changes between antigenicity, virulence, immunogenic potency, and colony morphology to differentiate and select smooth types from nonsmooth variants with altered characteristics on the basis of colony type (Braun: Bact. Revs., **11**, 75, 1947). This procedure is, however, none too objective and requires familiarity on the part of the investigator with the particular colony types. Such difficulties may be overcome by a recently discovered method that permits the automatic separation of antigenically smooth types from mixed populations containing both smooth as well as various nonsmooth types. This method was discovered in studies with *Brucella suis* and may be summarized as follows:

When one side of a U-tube, containing a fritted glass filter disk at the base is inoculated with a mixture of smooth and nonsmooth types suspended in saline or broth, only smooth types can be recovered from the uninoculated side 16 to 90 hours later. The U-tubes were made from commercially available pyrex straight tubes with centrally sealed fritted disk (total tube length 200 mm, diameter 13 mm, porosity of fritted disk "fine," maximum pore size 5 microns) by bending upward the glass tube extensions on either side of the fritted disk (figure 1). Two ml of broth or saline (NaCl concentration 0.85 per cent or more) were pipetted into each of the side arms of a U-tube, which was then sterilized. After the addition of as little as 0.04 ml of a suspension (40×10^9 cells per ml) containing both smooth and nonsmooth (e.g., intermediate, rough, and mucoid) types to one side of a U-tube, pure smooth cultures were isolated from the uninoculated side 16 to 90 hours later. Smooth types only could thus be isolated from the uninoculated sides of U-tubes seeded with populations containing as many as 99 per cent nonsmooth types. Inefficient separation in the presence of lower NaCl concentrations (0.5 per cent) could be overcome by the use of

larger inocula (0.5 ml). The cause for this separation of smooth types from mixed populations was found to be the clumping of nonsmooth types in saline and the inability of such clumps to pass through the fritted glass disk. Smooth type cells do not clump and are able to pass through the filter.

This method and device appears to be generally applicable for the simple separation of antigenically different types in all bacterial species that display linked variation in characteristics of antigenicity and salt sensitivity. Tests performed on *Bacterium tularense* with this U-tube (H. Eigelsbach, unpublished

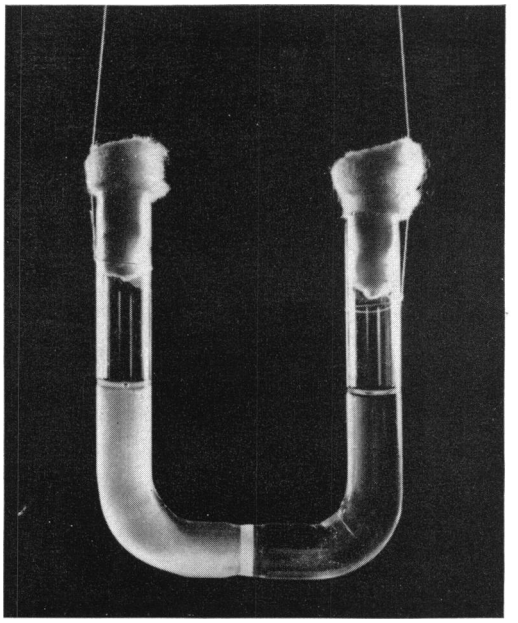

Figure 1. U-tube with fritted glass filter disk at the base.

data) confirmed that virulent smooth types of this organism could be separated with equal efficiency from mixtures containing approximately 10 per cent highly virulent smooth types and 90 per cent less virulent rough types.

TWO INTERMEDIATE MEMBERS OF ENTEROBACTERIACEAE

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Sachs (J. Roy. Army Med. Corps, **80,** 92, 1943) described ten strains of a mannitol-negative, aerogenic bacterium that was designated type A12. Wheeler

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