

of Froman *et al.* was used for the isolation of phage. Samples of soil, weighing 100 g, fertilized with human or chicken feces were taken into sterilized bottles and kept at 37 C. Four to ten day cultures of several different strains of saprophytic acid-fast bacilli and *M. avium* were suspended in distilled water after washing and were inoculated twice weekly. After six to ten inoculations the supernatant fluid was filtered through a Berkefeld bacterial filter and a drop of each of the soil filtrates was spotted on a 3 per cent glycerol agar plate which had been inoculated previously with each of the microorganisms used in enrichments. Phage dilutions of  $10^8$  particles per ml were used to determine the susceptibility of the organism.

As nine phage strains were obtained out of nine samples of soil, the efficiency of isolation was 100 per cent. The efficiency, however, was

very low when unfertilized soil was used as a source. Most of the phage strains isolated had a lytic activity against certain strains of virulent tubercle bacilli. Four phage strains were active not only against saprophytic acid-fast bacilli but also against human, bovine, avian, murine and so-called cold-blooded types of tubercle bacilli. The phage strain which had the broadest host range was named B1. The plaque type of B1 on host strain *M. avium* (Jucho) is shown in figure 1. B1 had a lytic activity against 8 out of 10 strains of human type, 1 out of 3 strains of bovine type, 3 out of 4 strains of avian type, 1 strain of murine type, 1 strain of so-called cold-blooded type of tubercle bacilli and 37 out of 57 saprophytic strains. An electron micrograph of an air-dried specimen of B1 is shown in figure 2. The heads were about 70 to 75  $m\mu$  in diameter and the tails were about 170 to 200  $m\mu$  in length.

## INDUCTION OF MOTILITY AND CAPSULATION IN *BACILLUS ANTHRACIS*

M. STERNE AND H. PROOM

*Wellcome Research Laboratories, Beckenham, Kent, England*

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The classical description of *Bacillus anthracis* includes the statement that the species is non-motile, this being one of the characters distinguishing it from other spore formers which it may superficially resemble. Since Smith *et al.* (U. S. Dept. Agr. Misc. publication, No. 559, 1946) classified *B. anthracis* as a variety of *Bacillus cereus* there have been several claims that motile anthrax strains have been observed or produced. Although we believe there are excellent reasons for assigning specific rank to *B. anthracis*, we are not concerned here with questions of taxonomy but of fact. To the clinical bacteriologist the absence of motility is important in the recognition of anthrax, and therefore reports that motile strains occur should be particularly well scrutinized, as they have repercussions extending beyond the limits of academic discussion.

Whenever we have encountered motile organisms in an anthrax culture we have been able to identify these as contaminants. Some of these could have been confused with *B. anthracis*, and to our knowledge have on occasion

been so confused. The following brief discussion explains why we have remained sceptical about reports on the occurrence of motility in *B. anthracis*.

Flewett (J. Gen. Microbiol., **2**, 325-333, 1948) mentioned the appearance of motile variants in ageing cultures of anthrax, but more recent observations on these "mutants" have convinced him that they were contaminants (Flewett, *personal communication*). Manninger and N6gr6di (Experientia, **4**, 276-277, 1948) described a motile, capsulated "mutant" derived from an avirulent strain of anthrax by the action of an extract from a capsulated *Bacillus mesentericus* (?). Tomesik (Schweiz. Z. Allgem. Pathol. u. Bakteriolog., **12**, 489-499, 1949) was unable to repeat this observation and found (Tomesik, *personal communication*) that Manninger's transformed strain and the *B. mesentericus* strain which provided the transforming principle were both *Bacillus subtilis*. Tomesik (Schweiz. Z. allgem. Pathol. u. Bakteriolog., **13**, 616-624, 1950) himself detected a motile capsulated organism closely resembling *B. anthracis* in a

culture of a mouse-virulent anthrax strain. He thought at first that this might be an anthrax mutant, but later decided that it was a *Bacillus megaterium* intermediate (Guex-Holzer and Tomcsik, *J. Gen. Microbiol.*, **14**, 14-25, 1956). We examined the culture and are in full agreement with this view.

This leaves the paper of Brown *et al.*, (*J. Bacteriol.*, **69**, 590-602, 1955) as the only recent and circumstantial description of motile anthrax mutants. These workers exposed anthrax cultures to a bacteriophage obtained from a virulent, lysogenic *B. anthracis* (strain Ohio). Nine of the bacteriophage treated strains—and only these nine—regularly produced motile variants which Brown *et al.* found biochemically and pathologically indistinguishable from *B. anthracis*. A careful study of the paper by Brown *et al.* revealed no obviously uncontrolled source of error. However, in their assessment of pathogenicity, the authors may have been unduly influenced by Nordberg (*Nord. Vet. Med.*, **5**, 915-924, 1953), who has apparently found difficulty in distinguishing experimental anthrax infections from deaths following the injection of broth cultures of *B. cereus* (Chu, *J. Gen. Microbiol.*, **3**, 255-273, 1949).

Dr. Cherry very kindly sent us 6 of the motile transformed strains; 4 proved to be *B. subtilis*, and 2 *B. cereus*. Our findings were reported to Dr. Cherry in September, 1955. He re-examined his stocks of motile *B. anthracis* and agreed that these were now contaminated and that no trace

of motile anthrax organisms remained. The matter was left in abeyance while Dr. Cherry tried to repeat the earlier work. These attempts have failed (Cherry, *personal communication*), and we feel that it should be made clear that the existence of motile *B. anthracis* is debatable, and that the ingenious genetic mechanism postulated for the derivation of these strains may be insecurely based.

In both the introduction and discussion to their paper Brown *et al.* placed considerable weight on the induction of motility and capsulation in an avirulent anthrax strain by Manning and N6grádi. However, the motile capsulated strain obtained was shown to be *B. subtilis* (Tomcsik, *personal communication*). Brown *et al.* also discussed at some length the derivation by Tomcsik (1949) of anthrax strains able to produce capsules under ordinary atmospheric conditions from strains which were unencapsulated under these conditions. Tomcsik actually stated that on the basis of experimental evidence the question of a transformation reaction has to be left open, and emphasized that he was unable to induce capsulation in a completely avirulent—that is to say, genotypically unencapsulated—anthrax strain.

It must therefore be emphasized that no transformation of *B. anthracis* from a genotypically nonencapsulated to a capsulated state has yet been effected. Arguments are therefore invalid if based on the impression that such transformations have been demonstrated.

## ADAPTATION OF THE MEMBRANE FILTER TECHNIQUE TO THE RECOVERY OF COAGULASE POSITIVE *STAPHYLOCOCCUS AUREUS* FROM HUMAN SALIVA

MARY L. SULLIVAN, H. E. LIND AND V. F. LISANTI

*Sias Research Laboratories, Brooks Hospital, Brookline, Massachusetts and Tufts University School of Dental Medicine, Boston, Massachusetts*

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The use of the membrane filter was initiated by Goetz and Tsuneiski (*J. Am. Water Works Assoc.*, **43**, 943, 1951) in bacteriological water analysis. It is presently being applied principally for the detection of *Escherichia coli*. There has been no report on the application of the membrane filter technique in the isolation and diagnosis of coagulase positive strains of

*Staphylococcus aureus*, particularly from samples of saliva.

This study was suggested in conjunction with a current investigation of the efficacy of direct inoculation of saliva onto glycine tellurite agar (Difco) as compared to Chapman Stone medium in the recovery of coagulase positive *S. aureus* strains. It was observed that the specificity of