

which phages were exposed failed to indicate any protective effect of "anticoagulants" against destruction of bacteria by heat (70°C.). Thus these findings suggest the possibility of employing heat in the presence of saccharose as a means of isolating phages from fecal cultures and from other natural sources instead of employing filtration for the purpose of removing bacteria.

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Effects of "K" Medium on the Filterability of Bacteria.

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Kendall employed a culture medium¹ consisting presumably of whole undenatured protein, devoid of all products of digestion which he referred to as peptones. He reported the transformation of ordinary bacteria into primitive forms capable of passing filters presumably impervious to the passage of normal bacteria.² Kendall concluded that he was dealing with unusual forms of bacteria, not only on the basis of their filterability, their appearance under the dark-field microscope, and their irregular staining, but also because of his inability to obtain sub-cultures by transplantation of the "filterable" forms to ordinary media, whereas sub-cultures were obtained by similar transplantation to his own, so-called K medium.

We here summarize some of our experiments to acquaint ourselves with this apparently new and important phenomenon.

The appearance of viable and cultivable bacteria in the filtrates of bacterial cultures has been repeatedly recorded. It is generally understood, that the mere passage of cultivable bacteria through filters does not predicate any radical change in their original size. It has been repeatedly shown that under a variety of conditions, normally effective filters may become permeable to bacteria of ordinary and even large size.

Even the first few experiments convinced us that in the case of cultures grown on K medium, the frequency of passage and the numbers of viable units of bacterial protoplasm appearing in the filtrates at each filtration were considerably greater than in the case

¹ Kendall, A. I., *Northwestern Univ. Bull.*, 1931, **32**.

² Kendall, A. I., *Northwestern Univ. Bull.*, 1931, **32**.

of cultures grown on ordinary media. Furthermore, the filterability of cultures grown in K medium was the greater the more profuse was the growth attained at the time of filtration. Increased growth could be secured by the addition of carbohydrates or peptone to the K medium and in either case the filterability was increased, thus it is evident that the exclusion of peptones from the medium is certainly not responsible for the increased filterability of Kendall's cultures.

A somewhat quantitative measure of the degree of filterability was secured by using the method of fractional filtration.³ The filtrates were collected aseptically into test tubes in portions of 10 cc. each, and the series of tubes thus secured were incubated. Appearance of growth in some of the tubes roughly indicated the point at which passage of bacteria began. It was found that growth appeared in the filtrates of cultures grown in K medium much sooner than in corresponding filtrates of broth cultures, apparently due to greater numbers of bacteria passing the filter in the first instance. It must be stated, however, that in all cases subcultures from filtrates of cultures grown either in K medium or in broth were successfully obtained by the transfer to either K medium or to plain broth.

Microscopic examination of cultures developing in K medium either before or after filtration invariably showed marked loss of motility of motile bacteria, with a gradual breaking up of bacterial cytoplasm into beaded forms and free granules, which exhibit very active Brownian movement but not true motility.

To determine the cause of the greater filterability of cultures grown on K medium, we suspended ordinary bacteria (grown on the surface of agar slants) in K medium and in plain broth respectively, and immediately subjected each type of suspension to filtration, allowing no lapse of time for growth and possible transformation of the bacteria to occur. Bacteria suspended in K medium passed the filter more readily than similar suspensions of the same bacteria in saline or plain broth, indicating a marked effect of the K medium upon the filter itself.

Kendall stated⁴ that only turbid K medium is suitable for the demonstration of filterable forms, and not the clear K medium. We found that turbid K medium is merely more effective in coating the filter bed. This renders the filters more permeable to bacterial sus-

³ Bronfenbrenner, J., and Muekenfuss, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1927, **24**, 371.

⁴ Public Lecture at Washington University.

pensions made either in K medium, in saline or in broth, when such suspensions are passed through filters just after passage of sterile K medium.

In addition to the substances in K medium which break down the efficiency of the filters, we thought that K medium might affect the filters because of its high fat content, since as much as 25% by weight of the dry powdered hog intestine base could be extracted by fat solvents. To determine this, we partially clogged filters by the repeated filtration of turbid K medium, and then extracted such filters with ether, alcohol and other fat solvents, and after washing retested their permeability to bacteria. We found that extraction with fat solvents did not restore the efficiency of the filters, whereas if they were cleaned in such a manner as to remove the adsorbed suspended matter, they were again rendered impervious to bacteria.

By inoculating K medium with several phages, Kendall was able to isolate staphylococci from staphylococcus phage. We inoculated sterile K medium, plain broth and blood agar with large inocula of 23 different phages prepared in this laboratory and known to be active. In no case but one was growth observed in the K medium cultures, and in this case growth was also secured in plain broth and on blood agar. The phage from which these cultures were made was known to be contaminated with a diphtheroid, the organism which developed in our cultures. Although transplants were made from the remaining K medium tubes at various intervals, absolutely no sign of growth occurred in such subcultures.

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Further Studies on the Mechanism of Transmissible Lysis of Bacteria.*

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Observing carefully the progress of lysis under the microscope we noticed that disappearance of individual bacteria under the influence of phage is usually preceded by more or less marked swelling

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