

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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of the cells.¹ A more detailed inquiry indicated that the swelling itself was due to imbibition of water which in turn appeared to be the result of intracellular digestion of bacterial cytoplasm,² and consequent increase of osmotic pressure within the cells. The actual disappearance of cells from the field was therefore interpreted as due to bursting.³ We found further that if bacteria are grown on media in which free water was immobilized by the addition to the

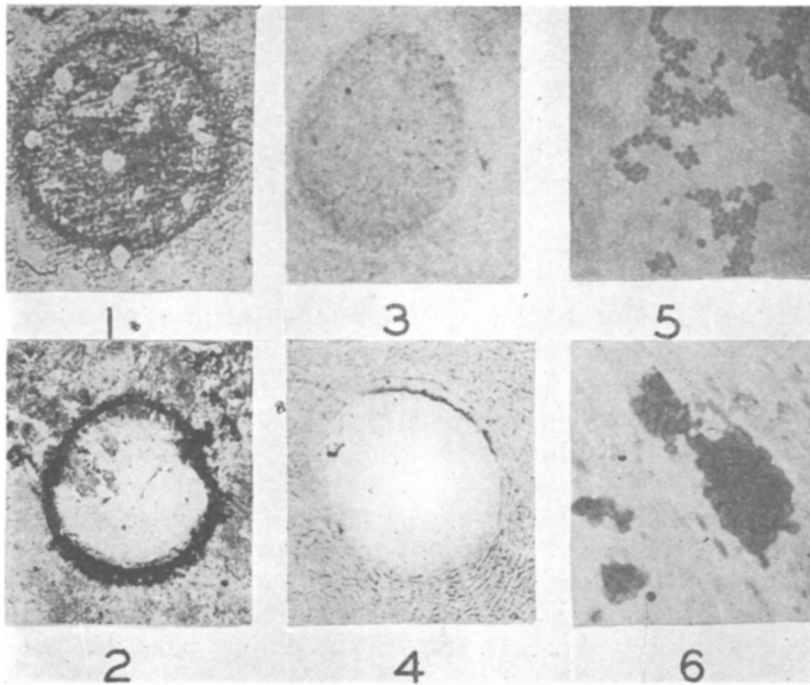


FIG. 1.

1. Stained "Klatsch" preparation showing increased growth of *B. coli* on 4% agar under the drop of bacteriophage. 25 $\times$.
2. Same showing lysis of *B. coli* on 1% agar under the drop of bacteriophage. 25 $\times$.
3. Direct photograph showing increased growth of *S. aureus* on 50% gelatin under the drop of bacteriophage. 25 $\times$.
4. Same showing lysis of *S. aureus* on 1% agar under the drop of bacteriophage. 25 $\times$.
5. Stained "Klatsch" preparation showing normal growth of *S. aureus* on 1% agar. 900 $\times$, enlarged three diameters.
6. Same showing swollen cells of *S. aureus* on edge of a plaque of lysis on 1% agar. 900 $\times$, enlarged three diameters.

¹ Bronfenbrenner, J., *PROC. SOC. EXP. BIOL. AND MED.*, 1926, **23**, 635.

² Hetler, D. M., and Bronfenbrenner, J., *J. Exp. Med.*, 1928, **48**, 269.

³ Bronfenbrenner, J., Muckenfuss, R. S., and Hetler, D. M., *Am. J. Path.*, 1927, **3**, 562.

medium of hydrophilic colloids, the swelling was prevented and consequently lysis did not occur. On the contrary, in place of a marked fall in the number of bacteria (as would be the case if lysis had occurred), we observed actual increase in the number of bacteria when compared with control cultures grown on the same medium but in the absence of phage.⁴ While the above observations were made with Gram-positive and Gram-negative bacteria, the changes were always more constant and more marked in the latter and therefore Gram-negative bacteria were used to illustrate the phenomenon.⁵ Recently there appeared a paper in which the authors,⁶ working with cultures of *Staphylococcus*, failed to observe either the swelling or the increased rate of growth of these organisms in the presence of phage. It was imperative to reinvestigate the subject.

The experiments here reported were performed to show that phenomena observed by us during the growth of Gram-negative bacteria in the presence of phage occur also with Gram-positive bacteria.

When either *B. megatherium*, *B. diphtheriae* or *Staphylococcus aureus* are grown on 4% agar or on 50% gelatine in the presence of phage, they all show increase in the growth rate as compared with that in control cultures grown under the same conditions but with broth added instead of corresponding bacteriophage. Under these conditions lysis does not take place. If, on the other hand, the same organisms are grown on ordinary media, the addition of phage causes swelling and lysis of organisms. The swelling in the case of Gram-positive bacteria is more difficult to record, however, than in the case of Gram-negative bacteria, because the cell membrane of Gram-positive bacteria in general is more permeable, hence the swelling never reaches the same degree as it does in Gram-negative bacteria before the bursting (lysis) takes place.

To obtain the material for demonstration of these phenomena, care must be exercised in securing proper conditions of the surface of the medium which would permit making the "Klatsch" preparations and in selecting suitable proportions between phage and bacterial inoculum so as to limit the lysis to a rate which would permit recording the changes.

⁴ Bronfenbrenner, J., and Hetler, D. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1928, **25**, 480.

⁵ Bronfenbrenner, J. See monograph on "Filterable Viruses", edited by T. Rivers, (Williams and Wilkins, 1927).

⁶ Krueger, A. P., and Northrop, J. H., *J. Gen. Phys.*, 1930, **14**, 223.