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## Inactivation of Staphylococcus Bacteriophage by Trypsin.*

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It is already known that bacteriophages active on intestinal organisms exhibit a high degree of resistance to the action of trypsin. With the exception of a trypsin-susceptible Shiga bacteriophage reported by Wollman,[1] this resistance seemed to extend itself to bacteriophages in general. Indeed, Arnold and Weiss[2] were able by trypsinization to free the lytic filtrates of bacterial and other proteinaceous residue. During the course of similar studies in the laboratory of d'Herelle at Alexandria, the writer had occasion to trypsinize a variety of lytic filtrates with the object of freeing them of these protein residues. In the course of this work it was discovered that 2 "races" of staphylococcus bacteriophage were rendered inactive by trypsin within a period of about 48 hours. The 2 "races" in question had been isolated by Dr. Gratia of the Pasteur Institute at Brussels. One of them appeared to be active only on one strain of staphylococcus, whereas the other was capable of lysing a number of staphylococcus strains. Both were highly active and produced complete clarification of homologous bacterial suspensions, and this better at 33° C. than at 37° C.

The experiments were originally carried out as follows: A small quantity (2-5%) of powdered trypsin† was emulsified in a freshly lysed culture and the treated filtrate was then filtered through coarse filter paper to remove the larger particles from the suspension. To this treated filtrate was then added about 2% of chloroform to inhibit bacterial growth. A capillary tube of heated egg-white was dropped into the mixture to serve as an indicator of the progress of tryptic activity. These suspensions were incubated at 37° C., the optimum temperature for tryptic digestion, for periods of 48 hours or longer. As a rule the chloroform served to keep the suspensions free of bacterial growth, so that it was possible in most instances to test the lytic activity of the trypsinized filtrate at frequent intervals without preliminary filtration through a Chamberland candle. The safety of this direct procedure in the case of tubes which gave no macroscopic evidence of bacterial growth was elicited by transferring several drops of the chloroform treated suspensions to

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† Several brands of trypsin have been employed.

broth alone. At times, however, the trypsinized suspensions treated with chloroform showed well defined bacterial growth, and curiously, this was often most marked immediately above the surface of the chloroform in the bottom of the tube. Chloroform sometimes also produces a peculiar flocculation in Martin's broth, which must not be mistaken for bacterial contamination. A number of tests were also carried out without the use of chloroform, but on filtrates which immediately after trypsinization were passed through filter paper and then through a Chamberland candle.

Under the conditions indicated, both "races" of staphylococcus bacteriophage appeared to be completely inactivated at the end of about 48 hours. Serial passages after this point failed to elicit any latent lytic activity. It is, however, important to carry the inactivation to its completion. When this is not the case the unlysed cultures are certain to reveal the lytic principle in its full potency in one or more serial passages. The nature and permanence of this inactivation by trypsin was not further determined. That it is not to be attributed to the chloroform is indicated by the fact that both bacteriophages were still active after 24 days exposure to chloroform alone. That it cannot be attributed to the action of trypsin on the staphylococcus used in determining the activity of the filtrate is indicated by the fact that these organisms grow abundantly in broth which has been recently trypsinized and filtered through a Chamberland candle. Moreover, the amount of trypsin, carried over into the bacterial suspensions from the trypsinized filtrate in conducting the test, is so small that it need not be considered. The possible influence of the trace of chloroform is ruled out by the analogous results with chloroform-free trypsinized filtrates.

A point of interest in connection with these studies is that freshly prepared staphylococcus suspensions in broth, which have received the trypsinized filtrate just before complete inactivation of the bacteriophage has been effected, may not show visible lysis in less than 48 hours or even 72 hours. Whereas control tubes having received the normal, untrypsinized lytic filtrate attain complete clarification within 24 hours; the tubes which had received the partially inactivated filtrate present at this time a heavy cloud of bacterial growth. The first impression one obtains is that the bacteriophage has at this point been definitely inactivated. That this need not necessarily be true is known to all students of bacteriophagy. A few serial passages may suffice to elicit a fully active bacteriophage from such unlysed cultures. The particular interest is that such heavily clouded unlysed cultures may begin to show complete lysis 48 to 72 hours later. In other words, these cultures do not begin to show clarifi-

cation until long after the period of most active bacterial growth has passed. The peak of bacterial growth is probably attained well within the first 24 hours. These observations certainly do not harmonize with the concept that the d'Herelle phenomenon is necessarily closely linked with the period of logarithmic bacterial growth. That this delayed lysis is not due to a spontaneous autolysis of the staphylococcus is indicated by the fact that nothing of the sort has ever been observed in control tubes containing the organism alone, or the organism plus a comparable amount of trypsin. Moreover, it is of interest that the lysis begins at the top of the turbid suspension and gradually extends downward as a sharply defined column of complete clarification. This may be easily followed in cultures undergoing lysis at room temperature. These observations are in harmony with the recent observations of d'Herelle[3] on the accelerating influence of increased oxygen tension on the lytic activity of the bacteriophage.

A study of the influence of trypsin on various coli, typhoid and dysentery bacteriophages failed to reveal any susceptibility on the part of these bacteriophages. Daily retrypsinization of these filtrates over a period of more than 10 days has left them active and true to form. No natural bacteriophage was recovered from several batches of trypsin examined. A single experiment on plague bacteriophage in Dr. d'Herelle's laboratory showed this bacteriophage inactive after five days exposure to trypsin. Because the supply of trypsin at this time became exhausted and none was immediately available, this experiment could not be repeated. On his return home, about 5 months later, the writer found that the plague bacteriophage had spontaneously become inactive, suggesting that this bacteriophage is not nearly as resistant as those we are more familiar with. It is well known that bacteriophages may be preserved over a period of years, at any rate, without losing their activity completely.

The writer is not inclined to predict that all staphylococcus bacteriophages may likewise be inactivated by trypsin. The literature on the bacteriophage teaches us the unwisdom of making generalizations on the basis of isolated observations. It will, however, be of interest not only to extend these studies to other "races" of staphylococcus bacteriophages, but to bacteriophages active for other nonintestinal microbes. The single experiment on the plague bacteriophage suggests that this property may possibly extend to other bacteriophages.

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[1] Wollman, E., *Compt. rend, Soc. de biol.*, 1924, xc, 59.

[2] Arnold, L., and Weiss, E., *J. Infec. Dis.*, 1925, xxxvii, 411.

[3] d'Herelle, F., *Compt. rend. Soc. de biol.*, 1927, xcvi, 451.