deavored by continuing the process to render the bacteriophages completely resistant to the action of trypsin we failed to get it beyond the point where merely a well defined difference could be recognized. We are not at all sure that the shift in property may in reality be regarded a true adaptation of the bacteriophage. Two such suspensions when tested some months later no longer exhibited the relative increase in resistance to the trypsin.

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Inactivation of Staphyloccus Bacteriophage by Methylene Blue.

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Recently, one of us[1] described the trypsin susceptibility of 2 races of staphylococcus bacteriophages, a property which stands in striking contrast to the marked resistance offered to this enzyme by bacteriophages generally. It occurred to us to compare these 2 races of bacteriophage with a number of other races in our possession, as to their susceptibility to other chemical agents, particularly dye stuffs. Various dyes, including Carmine, Congo Red, Methyl Red, Neutral Red, Methyl Green, Brilliant Green, Brilliant Cresyl Blue, Trypan Blue, Basic Fuchsin, Crystal Violet, Gentian Violet, Aniline Violet, Orange G, Eosin B, Bismark Brown, and Methylene Blue,* were added in relatively high concentrations to bacteriophage filtrates, which were then kept at incubator temperature, in part at room temperature, for 24 hours or longer and thereafter tested for lytic activity. No appreciable influence on any of the bacteriophages was noted, with the single exception of the effect produced on the 2 staphylococcus bacteriophages by methylene blue. These particular bacteriophages were completely inactivated within 6 to 12 hours by concentrations of the dye as low as 0.002%. Five serial passages on susceptible staphylococci failed to elicit any evidence of residual active principle. Believing that the action might be due to some impurities in the methylene blue, we then employed the dye after careful recrystallization. The same results were realized. Eight other bacteriophages, including races of anticoli,

[1] Schultz, E. W., PROC. SOC. EXP. BIOL. AND MED., 1928, XXV, 280.

* Mercks Medicinal Methylene Blue.

antidysentery, antityphoid, and antiproteus bacteriophages did not appear to be influenced in the least by the dye. The susceptibility to methylene blue appears therefore to be peculiar to the 2 races of staphylococcus bacteriophages examined for this property. This susceptibility may possibly extend itself to bacteriophages active for other non-intestinal bacteria as well as to other antistaphylococcus bacteriophages. The mechanism of the inactivation is not exactly clear, but will be studied further. We have been able to satisfy ourselves that it is not in any way related to pH changes induced by the dye in the medium.

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Spontaneous and Forced Dissociation of Brucella Abortus (Bang).

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In making a study, with Dr. J. Traum, on the causes of discrepancies occasionally encountered in testing cattle sera for *Br. abortus* agglutinins, it was noticed that different lots of antigens from certain strains gave varying reactions with the same sera. It was necessary to conclude that the antigen varied because all negative sera gave similar reactions on such lots of antigen and these same sera gave only definite negative results with antigens from many other strains of *Br. abortus*.

To determine the character of the variation mentioned above, glucose-glycerine agar plates were dallied with suspensions of the growth to be used for antigens. At the end of a 4-day incubation period, colonies of 2 distinct and constant types were present. One is the usual *Br. abortus* colony, moist, clear and only slightly granular. By transmitted light, these colonies show a blue-green florescence. The second type of colony is opaque, very definitely granular, and grows more rapidly than does the clear type. Subcultures from the clear type are readily suspended in salt solution and remain suspended for several days. The opaque type, on the other hand, is suspended with difficulty and spontaneously agglutinates within 24 to 48 hours. The subcultures of the opaque type have remained free of clear type colonies through several transplant generations. The clear cultures, however, continue to produce an occasional opaque colony even after several platings.