tion in media which regularly yielded spores of an enhanced resistance were not successful. Attempts to produce spores in any appreciable quantity in synthetic media prepared from highly purified chemicals met with failure.

Evidence gleaned from the literature and accumulated during the progress of this work supports the idea that the cause of death in cells exposed to a high temperature is the coagulation of bacterial protein. Conditions which render protein more difficult to coagulate consequently result in an increased resistance to heat. The water and the ash content of the cell appear to be especially important in this connection. However, cultivation under certain nutritive conditions which do not appear to be intimately related to either the water or ash of the cell has invariably resulted in the production of resistant spores.

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An Endeavor to Adapt a Trypsin Susceptible Bacteriophage to the Action of Trypsin.

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It is well known to students of the d'Herelle phenomenon that bacteriophages, with rare exceptions, resist the action of trypsin for indefinite periods of time. Recently, one of us[1] described the trypsin susceptibility of 2 races of staphylococcus bacteriophages; one a monovalent, the other a polyvalent race. These 2 races were found to be highly susceptible to the action of trypsin, complete inactivation following an exposure to this enzyme within 48 hours at incubator temperature. In view of the difference of opinion entertained by various investigators on the question of whether bacteriophages actually possess powers of adaptation, a property indicative of life, it occurred to us that this susceptibility to tryptic activity might possibly serve as a basis for determining further the capacity of bacteriophages to adapt themselves to deleterious or inhibiting agents.

Two methods of arriving at an answer were employed. In one series of experiments, the bacteriophage was exposed to the action

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

of trypsin in the presence of lysable staphylococci. That is, the customary procedure for producing bacteriophage suspensions was employed, with the exception that the Martin's broth contained active trypsin.* After complete lysis had been attained the limpid liquid was filtered through a Chamberland candle (L 3). A few drops of the filtrate were then added to a fresh suspension of staphylococci in trypsinized broth and so on in series. Any adaptation that might be effected could be due either to an adjustment on the part of the bacteriophage, or, should the bacteriophage in reality represent a product of the bacteria themselves, to an adjustment on the part of the staphylococci producing it.

In the other series, the bacteriophage was exposed to the action of trypsin immediately following filtration of the lysed culture through a Chamberland filter.* The exposure to trypsin therefore occurred in the absence of lysable staphylococci. After a variable period of exposure to the action of the trypsin, generally just before complete inactivation of the bacteriophage suspension, a few drops of the suspension were transferred to non-trypsinized Martin's broth containing an appropriate number of staphylococci for the regeneration of the lytic principle. The filtrates of these lysed cultures were again trypsinized to the point of almost complete inactivation; regenerated by passage on staphylococci in ordinary Martin's broth and so on in series. Any adaptation that might be realized would be more directly attributable to a shift in the properties of the bacteriophage itself. In both series suitable controls were always set up. Both series were repeated several times and were altogether carried on over a period of nearly a year.

The bacteriophages presumably undergoing an adaptation were tested at intervals for evidences of increased resistance to the action of trypsin. These measurements were perforce made in terms of the normal, "unadapted," bacteriophage. The criterion of adaptation consisted in noting any difference in the length of time that the bacteriophage, supposed to be undergoing adaptation, remained

* In preparing the trypsinized broth for the first series of experiments, also in trypsinizing the filtered bacteriophage suspensions in the second series, increasing quantities (1-4%) of powdered trypsin were added to the broth, or bacteriophage suspension, and after allowing the mixture to stand for about a half hour, was filtered through paper and finally through an L 3 Chamberland filter. The experiments were all carried out at incubator temperature and the activity of the trypsin was checked in each experiment by means of a capillary tube of heated egg-white immersed in the liquid. Several brands of trypsin were employed during the course of the work. No natural bacteriophages active for staphylococcus could be isolated from the trypsin preparations employed in the studies.

active after the normal bacteriophage, in the same set-up, had become definitely inactive. In carrying out these comparison tests every effort was made to secure reasonably comparable conditions for both the normal and "adapted" bacteriophages. The activity of these suspensions was determined at short intervals up to the time of complete inactivation. Suspensions were not considered completely inactive unless 5 or 6 serial passages on susceptible staphylococci failed to elicit any evidence of an active principle.†

The results obtained indicate that some increase in resistance to trypsin is acquired by the bacteriophage in the course of a number of serial exposures to suitable concentrations of trypsin. As nearly as could be determined, this increase in resistance was approximately the same for bacteriophages exposed to the action of trypsin in the absence of lysable staphylococci, as for bacteriophages regenerated at the expense of staphylococci, multiplying in the presence of an active trypsin. In neither of the series was the increase in resistance marked. Whereas the normal bacteriophages generally become definitely inactive by the end of 48 hours, the bacteriophages previously exposed to the action of trypsin remained definitely active at the end of this time and frequently continued to remain so for some days longer, provided retrypsinization was not resorted to in the meantime. Retrypsinization at the end of the 48 hour period always resulted in an inactivation of these more resistant suspensions within the next 24 hours. This may be explained on the ground that although some tryptic activity still manifests itself at the end of 48 hours, as judged by the continued digestion of egg-white in the capillary tube immersed in the bacteriophage suspension, the activity of the trypsin undoubtedly rapidly dwindles after this period. For this reason one cannot count bacteriophagic activity much in excess of 48 hours as evidence of further increase in resistance, unless retrypsinization of the suspension has been resorted to. Consequently, some increase in resistance could be elicited, *i. e.*, if we may make this deduction from a careful comparison with corresponding normal bacteriophages. Though we en-

† In this connection we invite attention again to the delayed lysis, previously reported by one of us,[1] in which clarification of the bacterial suspensions may not become obvious until some time after the period of logarithmic bacterial growth has ceased. We have been unable to elicit any evidence that the staphylococci undergoes spontaneous lysis, even when grown in trypsinized broth. The phenomenon is undoubtedly determined by a very low initial concentration of the bacteriophage. Such tubes when observed at room temperature first clear at the top and the clarification then gradually extends downward to the bottom of the tube.

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.