ment to large lots of "dormant" seed is so obvious as to require no emphasis.

With the establishment of a definite inhibitory action for blue light it became of interest to try to induce light-sensitivity (in this case also a so-called "dormancy") in normal or non-light-sensitive lettuce seed by subjecting moistened seed to blue light for a time and then drying it out. A series of such tests was carried out in which it was found that normal or non-light-sensitive lettuce seed could be made light-sensitive by subjecting it when moist to a strong blue light. Seed so treated would not germinate in darkness, but would germinate under red light, or by suitable exposure to red light and drying could be again rendered normal or "non-dormant" or non-light-sensitive. The reaction involved in the above procedure is thus reversible, but no attempts have been made as yet to localize or identify the material or materials responsible for the action.

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THE PREVALENCE OF STREPTOCOCCUS BACTERIOPHAGE

THE subject of bacteriophage, the lytic principle which destroys bacteria, is of special interest to bacteriologists, but it is of general interest to all biologists. Hence this report is offered to SCIENCE.

The general interest in bacteriophage lies in the hope that a solution of the problems in regard to its nature may contribute something to an understanding of the great mystery of life. The study of bacteriophage promises to enlighten the philosophical consideration because it stands at the border line between catalytic chemical substances, on the one hand, and living matter, on the other.

If bacteriophage be regarded as an enzyme it must be conceded that it is endowed with at least one of the attributes of living matter—a limited ability for adaptation to its environment. On the other hand, the minute size of the individual particles offers an obstacle to the acceptance of the idea that they may be living organisms. It has been shown that they may be no larger than certain protein molecules. They are so small that ten or even a hundred billion individuals may exist in a cubic centimeter of broth which nevertheless remains as clear as crystal. In this communication no attempt is made to add weight to either side of the argument as to whether or not bacteriophage should be regarded as living matter, except in so far as new information on one particular aspect of bacteriophage may contribute to a general understanding of the subject.

Although it is known that bacteriophage specific for intestinal bacteria may be readily isolated from sewage, the belief is common that streptococcus bacteriophage is rare and difficult to obtain. In his recent review of the literature on bacteriophage Burnet[1] concludes that it is extremely rare to obtain a phage active against streptococci. He mentions the race which was isolated by Clark and Clark[2] as the only indubitable streptococcus phage on record. It will be referred to here as the "Wisconsin" phage.

The writer studied the streptococcus phage in commercial preparations and found that a second race has been distributed to various laboratories in this country, although it has been merely mentioned in the literature.[3] This second race was isolated in 1928 by Dr. Pearl Kendrick, of the Michigan Department of Health, from the feces in a case showing intestinal hemorrhages. It will be referred to as the "Michigan" phage.

A third race was isolated by the writer in April, 1933, from a sample of sewage received from Cincinnati. This race, designated as the "Cincinnati" phage, was recently reported briefly.[4]

The 3 phages were found to be useful in a study of the relationships of hemolytic streptococci. The collection on which this study is being made consists of over 300 strains from wide geographical sources and from all kinds of streptococcus infections of man and domestic animals. Some of the strains were found to be sensitive to one of the races of phage, some to another and some were sensitive to two or all three of them. In general, when several strains from a given epidemic were available, they were found to agree in their sensitivity to the 3 phages. Thus it became apparent that sensitivity or resistance to several races of phage offers a characteristic useful for the identification of hemolytic streptococci.

One rather large group of streptococci was characterized by failure to show sensitivity to any except the "Wisconsin" phage, which under certain conditions is capable of attacking about 97 per cent. of strains of human origin. It seemed desirable to find another race of phage which might attack the strains of this group, in order to give the group a more distinctive character. The readiness with which streptococcus phage was found in the samples of sewage examined is so contrary to its reported rarity that it seemed worth while to record the observations.

The group of streptococci for which a phage was

[1] F. M. Burnet, "Bacteriophage and Cognate Phenomena," in "A System of Bacteriology," Vol. 7, London, pp. 463–509, 1930.

[2] Paul F. Clark, and Alice Schiedt Clark, "A Bacteriophage Active against a Virulent Hemolytic Streptococcus," *Proc. Soc. Exp. Biol.*, 24: 635–639, 1927.

[3] Pearl Kendrick and Harriet C. Hollon, "Serologic and Bacteriophagic Relationships in a Group of Fecal Streptococci," *Jour. Bact.*, 21: 49–50, 1931.

[4] Alice C. Evans, "Streptococcus Bacteriophage and Its Usefulness for the Identification of Strains of Hemolytic Streptococci," *Ibid.*, 27: 49–50, 1934.

sought contained, among others, strains from several epidemics of septic sore throat which occurred in Massachusetts and Connecticut within recent years. Therefore it was logical to search for the desired phage in sewage from cities of those states during the season when streptococcus infections are prevalent. I am indebted to the health officers of Boston and Hartford for the two samples of sewage examined. Both were received in January, 1934. The examination of the two samples was made simultaneously.

TECHNIQUE

The technique was similar to that commonly employed to obtain from sewage phages active against other species of bacteria. The medium was meat infusion broth of double strength, which was distributed in test tubes, sterilized by heat and then diluted with an equal quantity of sewage from which all bacteria were removed by filtration. To this medium 25 per cent. of sterile filtrate was added from cultures as described below.

For the first culture generation of streptococci the filtrate added to the sewage medium was from culture planted with the unfiltered sewage, and incubated overnight at 37° C. The inoculum for this first culture generation of streptococci was with a mixture of 10 strains. A very light inoculum was prepared by adding one drop of over-night culture of each of the strains to a tube containing about 10 cubic centimeters of broth, and planting one drop of this diluted mixed culture in the sewage medium. After growth over night the culture was filtered, and the filtrate was tested to demonstrate any lytic principle it might contain by planting each of the 10 strains in pure culture into broth containing 10 per cent. of the filtrate.

For the second and following culture generations in the sewage medium inoculations were made with the 10 strains in pure culture, while the filtrate added to the medium was a mixture of filtrates from the 10 preceding pure streptococcus cultures. The lot of 10 streptococci was made up of representative strains from the group for which a new phage was sought.

A weak lytic principle appeared in the filtrate of the third culture generation in the medium containing the Boston sewage. The procedure was continued, using now only 3 or 4 of the most sensitive streptococcus strains of the lot. It required only a few more such passages to attain what appears to be the maximum potency for this race of phage—the ability to lyse sensitive strains in a dilution of about 1 to 10^6.

This new race, designated the "Boston" phage, is not neutralized by any one of the 3 serums specific for the Wisconsin, Michigan or Cincinnati races of phage. (Anti-phage serum is prepared by treating rabbits repeatedly with the phage for which the antibody is desired.) Hence there are now 4 antigenically distinct races of streptococcus bacteriophage available for study.

Both the Boston and the Hartford samples of sewage were further examined for the presence of a phage similar to the Cincinnati race. The procedure was as outlined above, except that the 10 selected strains of streptococci were all sensitive to the Cincinnati phage. A lytic principle appeared in the filtrates from the first generation of the mixed streptococcus culture in both the Boston and Hartford media. Both lysates were active against the same strains of streptococci as the Cincinnati phage, and both were neutralized by the serum of a rabbit treated with the Cincinnati phage. According to these criteria both races are identical with the Cincinnati phage.

The readiness with which bacteriophage was obtained from the Boston and Hartford samples of sewage indicates that streptococcus bacteriophage is widely distributed, at least during the season when streptococcus infections are prevalent.

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EFFECTS OF PROTRACTED EXPOSURE TO A LOUD TONE[1]

WITHIN recent years, Yoshii and others have published evidence which purports to show that protracted exposure of animals to intense tonal stimulation results in localized cochlear lesions whose foci are dependent upon the exposure-frequencies.[2] More recently, Upton attempted to determine the functional effects of exposing guinea pigs to a 1,000-cycle tone, and reported marked loss of acuity to the exposure frequency but no loss at 500 cycles.[3] These results have been widely accepted and are commonly interpreted in favor of the resonance-hypothesis. The methods employed do not always carry conviction, however, particularly in the crucial matter of measuring what the animal actually can, and can not, hear. The indicators by which the animal reveals that it hears a given tone (usually some form of startle-behavior, *e.g.*, pinna- or breathing-reflex) are notoriously subject to extinction by the very condition of long-continued exposure to that tone; hence we

[1] Communication No. 5 from the Alpha Research Laboratory, Department of Psychology, established and maintained with aid from the Research Trustees, American Otological Society. Special aid in this problem from the Elizabeth Thompson Science Fund is gratefully acknowledged.

[2] For references, see C. v. Eicken, *Abderhalden's Handb. d. biol. Arbeitsmethoden*, v: 771, 1929.

[3] M. Upton, "Functional Disturbances of Hearing in Guinea Pigs after Long Exposure to an Intense Tone," *Jour. General Psychol.*, 2: 397–412, 1929.