SPECIAL ARTICLES

THE PRESERVATION OF A POLYVALENT STAPHYLOCOCCUS BACTERIOPHAGE

FOR a period of several months we have been engaged in preparing polyvalent staphylococcus bacteriophage for use in a clinic in a large Eastern hospital, and for investigative work in this laboratory. Obviously a bacteriophage prepared for clinical use should be a very polyvalent one and in addition should be free from contamination and fear of secondary growth. We have kept these two factors in mind constantly.

At various times we have purchased staphylococcus bacteriophages prepared by commercial laboratories and compared the potency of their products with that which we are preparing. One of these samples—a semi-solid preparation—was found to inhibit the growth of many strains of staphylococci as well as streptococci, *B. coli* and *B. dysenteriae*. This inhibiting action was not due to the presence of a strong polyvalent phage, but to an antiseptic, since the inhibiting substance was not transmissible in series and it was not destroyed at 80° C. The protocols of these experiments are recorded elsewhere.[1]

Jamieson and Powell[2] have reported that Merthiolate in a concentration of 1-5,000 may be "used as a preservative for highly potent bacteriophage with little detectible damage." These authors also state that "dense suspensions and broth cultures of bacteria may be rendered sterile by exposure to 1-5,000 to 1-10,000 Merthiolate concentrations for a few days in the ice box." It is hardly possible that the same concentrations of an antiseptic such as Merthiolate which renders bacterial cultures sterile would not have the same effect upon bacteriophage. The studies of Clifton[3] indicate that a concentration of 1-10,000 of Merthiolate in staphylococcus bacteriophage incubated at 37.5° C. for 30 days markedly decreases the activity of the phage. Under the same conditions a concentration of 1-100,000 of the antiseptic has very little effect.

A sample of Merthiolate (Solution No. 45 Merthiolate, 1-1,000) was purchased and concentrations of 1-10,000 and 1-100,000 added to a polyvalent staphylococcus bacteriophage. Titrations of the bacteriophage Merthiolate suspensions were made after two hours, at room temperatures; and after five and ten days at incubator temperature (37.5° C.). The strain of staphylococcus used as a test organism was lysed by this polyvalent phage in a dilution of 10^{-7}. The results are given in Table I.

TABLE I

Incubation	Temp.	Lysis in dilutions of		
2 hours	20° C.	Bp 8	Bp 8,1: 10,000 Merth	Bp 8,1: 100,000 Merth
		$4+10^{-7}$	$4+10^{-3}$	$4+10^{-5}$
5 days	37.5	$4+10^{-3}$	$4+10^{-3}$	$4+10^{-2}$
10 days	37.5	$4+10^{-2}$	$4+10^{-3}$	$4+10^{-2}$

4+ = complete clearing in test-tube.

It appears from these results that a bacteriophage loses a good deal of its potency when it is incubated at 37.5° C. for several days. This phenomenon has been observed by d'Herelle, Sertic[4] and others and we have observed it repeatedly. However, a strong bacteriophage kept at room temperature maintains its initial high virulence for months and even years. Bacteriophage 8 was prepared early in February and is still as active as it was originally. Merthiolate very quickly exerts an injurious effect when mixed with bacteriophage in concentrations of 1-10,000 and 1-100,000, and after two hours' contact one may observe a reduction in potency of the bacteriophage. After five days' incubation the bacteriophage Merthiolate suspensions inhibit the action of the growth of staphylococcus but this is due to the action of the antiseptic, for the same results may be obtained when these tubes are heated at 87° C. for 12 minutes. Furthermore, no plaques were ever observed with the same dilutions of phage antiseptic that inhibited growth in the test-tube. The tube containing bacteriophage without the addition of Merthiolate was killed at the above mentioned temperature, and no action against a susceptible staphylococcus could be observed. Our results indicate that Merthiolate in dilutions up to 1-1,000,000 quickly and permanently destroys bacteriophage.

All staphylococcus bacteriophage prepared in this laboratory is filtered through L5 Chamberland candles, and each candle is heated to red heat in a muffle furnace before it is used again. The polyvalent bacteriophage prepared for the clinic is filtered into a previously sterilized, highly efficient ampoule machine devised by d'Herelle. Every ampoule is subjected to temperatures of 56 to 57.5° C. for one hour for three consecutive days. Between the periods of heating the ampoules are incubated at 33° C. There is no decrease in the potency of a bacteriophage treated in this manner. Furthermore, we have never observed any evidence of growth in the ampoules. Each ampoule is tested for evidence of possible contamination after the final heating by the use of an artificial light (Tyndall effect). Samples of the phage

[1] In press.
[2] *Am. Jour. Hyg.*, 14, 1931, 218.
[3] *Proc. Soc. Exp. Med. and Biol.* 29, No. 4, 1932, 370.
[4] Personal communication.

are also inoculated into anaerobic medium and incubated for at least one week.

CONCLUSIONS

Bacteriophage may be prepared and kept for long periods of time without fear of secondary cultures.

No antiseptic need be added.

Merthiolate destroys the action of a staphylococcus bacteriophage within a period of five days at a temperature of 37.5° C.

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EFFECT OF THALLIUM ON GROWTH OF TOBACCO PLANTS

IN view of the recent interest in thallium poisoning[1, 2] the following preliminary report of the effect of this element on the growth of tobacco is given at this time. The previous papers[3] dealing with the effects of this element report the death of the plant, but no mention appears to have been made as to its effects when present in smaller amounts than that causing death. In recent years an extensive search has been conducted as to the cause and the control of the so-called frenching disease of tobacco. Valleau and Johnson[4] have reported that this disease is due to nitrogen deficiency, but our results have not substantiated their findings. In connection with a study of this disease trials were made of the toxicity of several of the heavy metals as possible causes of the trouble. Thallium in the form of the nitrate was included in this study and produced very decided effects on the growth of the tobacco plant. The element was applied in pot cultures at 35 and 75 p.p.m. based on the air dry weight of the soil after the plants had become established. Three typical sandy loam tobacco soils were used, two of which were held at two moisture contents. The severity of the effects of thallium differed to some extent in the different soils and was greater at the higher moisture contents. In many instances the stem was killed at the surface of the soil. This effect may have been due to the fact that the material was applied in solution and was not leached to any considerable extent from the surface layers. Thallium, when applied as the nitrate, was not leached from an inverted bell jar containing soil, to a pot below which was watered only by the leachings from the bell jar, in sufficient quantity to affect a tobacco plant growing in the pot, indicating that this element is not readily leached from the soil. This point is being tested further, and it is possible that much smaller quantities of this element will prove toxic if evenly distributed through the soil mass.

In solution culture tests one part per million of the element thallium has been found to produce the following described effects. Where the plant is not killed outright thallium toxicity is manifested by a series of effects which are characteristic symptoms of frenching, though the two are not identical in all particulars. The first effect is a slowing down of the growth rate and the development of a lighter green color along the veins of the upper leaves of the plant. The younger leaves as they develop show at first a chlorosis following out the smallest branches of the vascular system, but this chlorosis does not so characteristically originate at the tip and margins of the leaf as is the case with typical frenching. The subsequent growth produces leaves which are decidedly distorted and in many instances may consist essentially of only the midrib. This is followed by a proliferation of the lateral buds resulting in the so-called witches broom effect. These later stages agree very closely with the symptoms of typical frenching, which has been observed so widely in the field on practically all soil types where tobacco is grown but usually only on small local areas in any given field.

Whether the typical frenching disease of tobacco is due to thallium toxicity can not be definitely stated at this time, but it appears that there is much in common in growth manifestations exhibited by the two pathological conditions. It is at any rate a matter of considerable interest to recognize the striking effects on plant growth produced as a result of the toxicity of this element.

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[1] S. C. Brooks, "Thallium Poisoning and Soil Fertility," SCIENCE, n. s., 75 (1934): 105–106, 1932.

[2] Marcus Ward Lyon, Jr., "Thallium Poisoning," SCIENCE, n. s., 75 (1945): 381–382, 1932.

[3] W. Knop, Ueber die Aufnahme Verschiedener Substanzen durch die Pflanzen Welche Nicht zu den Nahrungstoffen gehoren. K. Sächsische Gesellschaft der Wissenschaften Berichte ueber Verhandlungen. Math-Physiche Classe, 35–37, 1883–1885.

[4] W. D. Valleau and E. M. Johnson, "Tobacco Frenching A Nitrogen Deficiency Disease," Kentucky Agr. Exp. Sta. Res. Bul. 281, illus., 1927.

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