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SHORT COMMUNICATION

The effect of peptone on the inactivation of a bacteriophage by chemical antimicrobial agents

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BECAUSE of their intracellular parasitic nature, infectious virus particles are almost invariably closely associated with substances derived from the host organism. In the chemical disinfection of virus contaminated material, the possible interference in the action of the inactivating agent by extraneous organic matter is therefore of particular importance. Little quantitative information on such effects is available however. Peptone reduces the activity of formaldehyde, phenol and sodium hypochlorite against *Bacillus subtilis* spores (Bullock & Rawlins, 1950, 1954) and it has been used here to illustrate the effect of non-viral organic matter on the chemical inactivation of a bacterial virus.

The bacteriophage (coliphage T6r) and the host bacterium (*Escherichia coli*) were cultivated as previously described (Cook & Brown, 1963). Plaque counts were made by the soft agar layer method (Adams, 1959; Brown, Cook & Oduro-Yeboah, 1964). All phage inocula were taken from a single suspension in peptone water (1·0% peptone and 0·5% sodium chloride) containing approximately 1×10^9 plaque forming units (p.f.u.) per ml and stored at 4°.

Oxoid Peptone (L37/1881A) was used throughout, a sterile aqueous solution containing 2·0% peptone and 0·5% sodium chloride being diluted as required with 0·5% aqueous sodium chloride solution. The antimicrobial agents were cetrimide B.P., chloramine-T (B.D.H. Laboratory Reagent), formaldehyde solution (Analar) and phenol (Analar).

Inactivation of the phage by formaldehyde in the presence of 0·01% peptone follows the kinetics of a first order chemical reaction while inactivation by cetrimide and chloramine-T shows deviations from first order kinetics similar, though not identical, to those reported for phenol (Brown, Cook & Oduro-Yeboah, 1965). Details of the time-survivor curves obtained will be published elsewhere. For the present work, concentrations of the antimicrobial agents were used which, in the presence of 0·01% peptone, gave at least 90% inactivation of the inoculum in a fixed time of exposure (30 min) at 25°. The effect of peptone was tested therefore on the resistant fraction of the inoculum which survived the initial rapid inactivation by cetrimide, chloramine-T and phenol.

Reaction mixtures were prepared by adding 0·9 ml peptone solution of appropriate concentration and 0·1 ml phage suspension to 9 ml aqueous solution of the antimicrobial agent. After 30 min at 25°, samples were diluted with a suitable neutralising agent and 6 replicate plates prepared for each dilution. Each test was performed in triplicate. Reaction mixture samples containing phenol were diluted in peptone water; those

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containing cetrimide, chloramine-T and formaldehyde were diluted with peptone water containing 3·0% Tween 80, 1·0% sodium sulphite and 1·0% dimedone-morpholine respectively. Initial dilutions were made at least 1 in 10 and subsequent dilutions, when required, were prepared with peptone water in every case. Except where otherwise indicated, the samples were plated immediately after dilution. This procedure had been shown previously to neutralise effectively the antimicrobial agents.

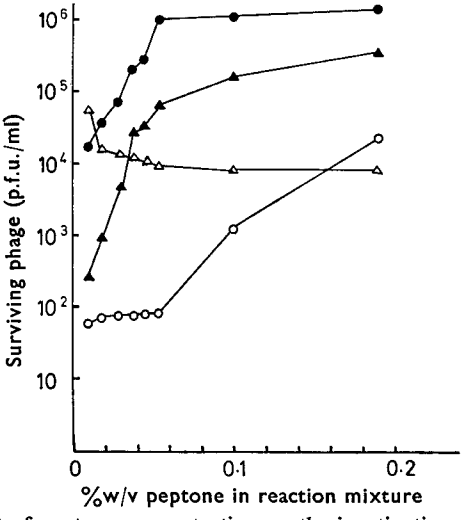

FIG. 1. The effect of peptone concentration on the inactivation of coliphage T6r in 30 min at 25° by cetrimide, $7{\cdot}2 \times 10^{-3}$% w/v ○—○. Chloramine, $3{\cdot}6 \times 10^{-2}$% w/v ▲—▲. Formaldehyde, $3{\cdot}6 \times 10^{-1}$% w/v △—△. Phenol, 2·25% w/v ●—●. Phage inoculum $1{\cdot}68 \times 10^{7}$ plaque forming units (p.f.u.) per ml. Each point represents the mean of 3 replicate tests.

Phage suspensions in 1% and 0·01% peptone solutions without antimicrobial agents, of the same titre as the inocula of the reaction mixtures, showed no change in plaque count during 14 days at 25°.

The effect of peptone concentration on the inactivation of the phage by one concentration of each antimicrobial agent is shown in Fig. 1. The minimum concentration of peptone tested (0·01%) was that resulting from the peptone present in the phage inoculum and, for the present work, was regarded as the control preparation.

Cetrimide, chloramine-T and phenol showed a *decrease* in viricidal activity as the peptone concentration *increased*. The effect on phenol was most pronounced up to 0·055% peptone whereas with cetrimide it became significant only above this concentration. Further work is in progress to elucidate the significance of this concentration of peptone.

Formaldehyde showed an *increase* in activity with increased peptone concentration, an effect which is contrary to the usual effect in the inactivation of bacteria. A possible explanation is suggested by the results obtained when diluted samples of formaldehyde treated phage were stored at 4° and 24° and plaque counts performed at intervals during 5 hr (Fig. 2). The recovery of phage from reaction mixtures containing 0·01% peptone increased markedly with time of storage, the increase being more

pronounced at the higher temperature tested. No such increase in recovery was found in samples from reaction mixtures containing 0·1% peptone. Some of the phage particles treated with formaldehyde in the presence of low peptone concentrations are, therefore, reversibly inactivated as has been reported by Schultz & Gebhardt (1935) for a staphylococcus phage and by Heicken & Spicher (1959) for coliphages. In the presence of higher concentrations of peptone the phage are permanently damaged, possibly by the formation of a formaldehyde-peptone complex which is held at the site of action more firmly than formaldehyde alone.

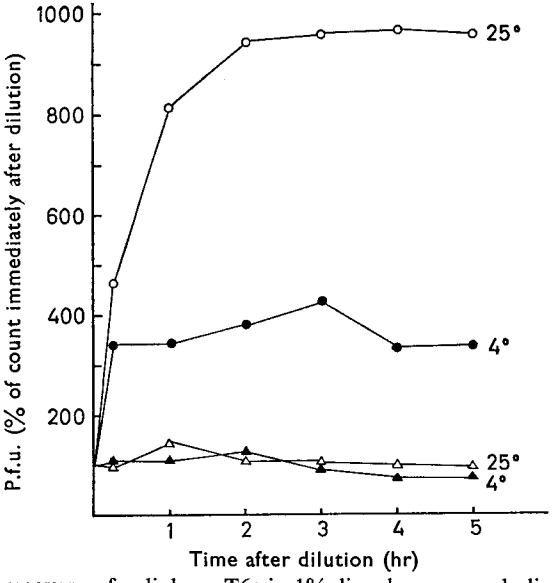

FIG. 2. The recovery of coliphage T6r in 1% dimedone - morpholine at 4° and 24° after treatment with formaldehyde ($3{\cdot}6 \times 10^{-1}$% w/v for 30 min at 25°) in the presence of peptone. Counts expressed as % of count immediately after dilution (ca. 99·9% inactivation of original inoculum). Each point represents the mean of duplicate tests. 0·01% peptone in reaction mixture ○, ●. 0·10% peptone in reaction mixture △, ▲.

Samples from reaction mixtures containing cetrimide, chloramine-T or phenol and 0·01% peptone showed no alteration in recovery with time after dilution.

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