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Effect of concentration and temperature on the inactivation of a bacteriophage by phenol

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The concentration of phenol affects not only the rate of inactivation of phage but also the shape of the survivor-time curves. The shape of the curves obtained indicates that phenol may inactivate phage in two suggested stages involving different components of the phage protein. The concentration exponent for the inactivation depends on the level of inactivation chosen. The temperature coefficient is independent of the level of inactivation. The temperature of incubation of phenol treated phage does not obviously affect the recovery of the phage.

A NUMBER of published reports of studies of the inactivation of viruses have shown that the rate of inactivation may not follow the kinetics of a first order reaction. Gard (1960) and Hiatt (1964) have commented on the possible significance of the shapes of inactivation curves for viruses but little information is available on the effects of the concentration of inactivating agent and the temperature of the reaction on the kinetics of virus inactivation. The effects of these factors on the inactivation of a coliphage by phenol have now been investigated.

Experimental and results

The host organism (*Escherichia coli*) and the bacteriophage (Coliphage T6r) were cultivated by the methods described by Cook & Brown (1963). Inactivation of the bacteriophage was investigated by the procedure previously described (Brown, Cook & Oduro-Yeboah, 1964).

EFFECT OF CONCENTRATION OF PHENOL

Bacteriophage inocula for all the reaction mixtures were taken from the same phage stock (stored at 4°) which had an initial phage titre in the reaction mixtures of approximately $2{\cdot}5 \times 10^7$ phage particles per ml. The number of surviving infective phage particles was estimated by the surface drop method of counting. The course of the inactivation of the bacteriophage by various concentrations of phenol in aqueous solution at 25° (± 0·05°) are shown on a semilog plot in Fig. 1A. Fig. 1B shows the same results on a log-log plot. The relationship between log contact time and log phenol concentration for 90·0%, 99·0% and 99·9% inactivation (Fig. 2A) was linear ($r = 0{\cdot}9947$, 0·9769 and 0·9793 with degrees of freedom of 4, 4 and 3 respectively) and the calculated slopes (or concentration exponents) for the regressions were −8·628, −11·628 and −13·164 respectively.

INFLUENCE OF TEMPERATURE OF REACTION

Inactivation of the bacteriophage by 2·0% (w/v) aqueous phenol at various temperatures was investigated using a bacteriophage stock which

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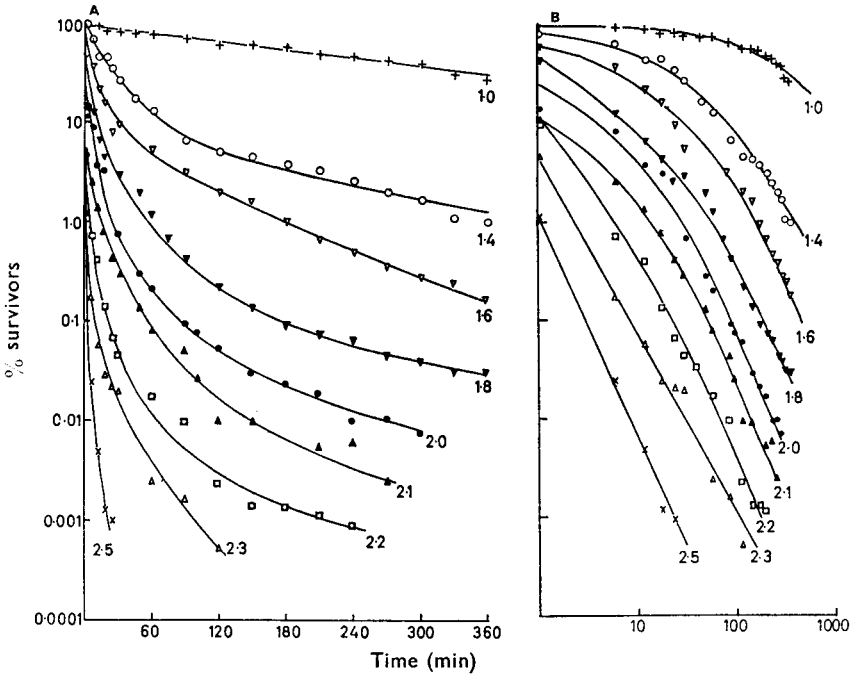

FIG. 1. Inactivation of coliphage T6r by phenol (25°). A, log% survivors plotted against time. B, log % survivors plotted against log time. Figures on curves are % concentrations of phenol.

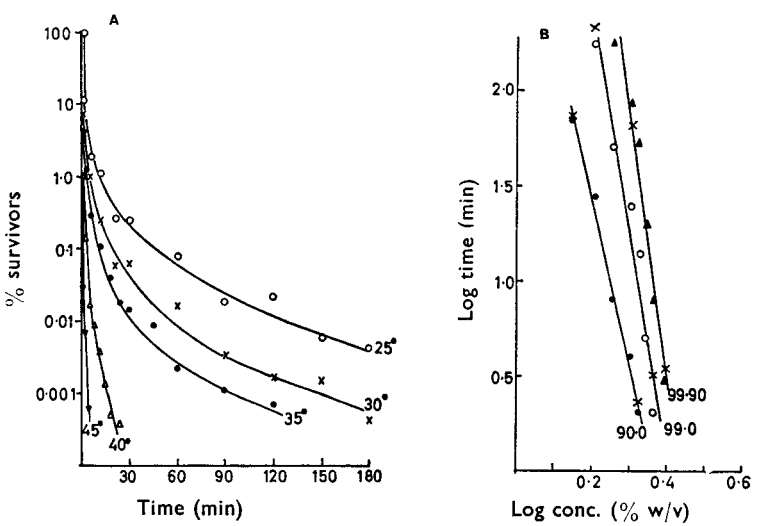

FIG. 2. A. Relationship between log contact time and log concentration of phenol for different levels of inactivation of coliphage T6r (25°). (×——×, calculated regression of log contact time upon log concentration of phenol). Figures on curves are % kill. B. Influence of temperature on inactivation of coliphage T6r by 2·0% phenol. Figures on curves are temperature °C.

had an initial titre in the reaction mixtures of approximately 6×10^7 phage particles per ml. Surviving phage particles were estimated by the soft agar layer method of counting. The reaction mixtures were maintained within $\pm$ 0·05° of the required temperature and the results are illustrated in Fig. 2B. Using the contact times required to effect 99, 99·9, 99·99 and 99·999% inactivation, the temperature coefficient (θ) for the inactivation of the phage was calculated from the formula $\theta^{T_1-T_2} = \frac{t_1}{t_2}$, where t_1 and t_2 are the times producing the required % inactivation at temperatures T_1 and T_2 respectively (Table 1).

TABLE 1. TEMPERATURE COEFFICIENTS (θ) FOR THE INACTIVATION OF COLIPHAGE T6r BY 2% W/V PHENOL CALCULATED FOR VARIOUS % INACTIVATION

Temperature range, °C	Temperature coefficient (θ) for % inactivation of phage			
	99	99·9	99·99	99·999
25–30	1·15	1·17	1·17	—
30–35	1·13	1·12	1·12	1·09
35–40	1·18	1·32	1·33	1·41
40–45	1·21	1·34	1·32	1·28

EFFECT OF THE TEMPERATURE OF INCUBATION DURING RECOVERY

Duplicate experiments were performed for the inactivation of the phage by 2·0% (w/v) phenol at 25° and 30°. For each reaction temperature one set of plates was incubated at 37° and the other set at 26°. The results are shown in Table 2.

TABLE 2. EFFECT OF THE TEMPERATURE OF INCUBATION ON THE RECOVERY OF COLIPHAGE T6r AFTER EXPOSURE TO 2% W/V PHENOL AT 25° AND 30°

Temperature of reaction mixture °C	Contact time (min)	% surviving phage at incubation temperature of °C	
		26	37
25	0	100·00	100·00
	30	0·139	0·080
	60	0·076	0·061
	120	0·025	0·018
	180	0·0010	0·0012
30	0	100·00	100·00
	12	0·256	0·202
	30	0·053	0·054
	60	0·016	0·023
	120	0·0026	0·0017

Discussion

The concentration of phenol to which the phage was exposed had a marked effect on the kinetics of the inactivation of the phage. The lowest concentration of phenol tested (1% w/v) showed a rate of inactivation which followed the kinetics of a first order chemical reaction. Increasing the concentration of phenol produced an increase in the initial rate of

inactivation; the rate became less as the time of contact increased. That fraction of the inoculum undergoing this rapid inactivation also increased as the phenol concentration rose until at 2·3 and 2·5% w/v the relationship between log % survivors and log contact time was linear. This relationship has previously been shown to persist, at least for 2·5% w/v phenol, until the inactivation of the phage is virtually complete (Brown & others, 1964).

Inactivation of poliovirus by formaldehyde shows a deviation from first order reaction kinetics similar to that shown here for concentrations of phenol of 1·4–2·2% w/v. Salk & Gori (1960) attributed the shape of the inactivation curve partly to the high resistance of a small fraction of the viruses present and partly to the aggregation of virus particles during the course of the reaction. They suggested that particles embedded in an aggregate were protected from inactivation. Gard (1957, 1960) suggested that the fall in the inactivation rate resulted from the formaldehyde causing hardening of the protein coat of some of the virus particles. Such hardening reduced the permeability of the protein and the rate at which formaldehyde can reach the vital deoxyribonucleic acid (DNA) core of the particle. Heicken & Spicher (1956) arrived at similar conclusions for the inactivation of the T-even coliphages with formaldehyde.

It is possible that the shape of the inactivation curves for coliphage T6r by phenol is due to effects similar to those suggested for formaldehyde. However, the effects of different concentrations of phenol on the shape of the curves suggest that the shape reflects the mode of action of the phenol on the phage and that at least two stages are involved. One stage occurs at low concentrations of phenol and gives a rate of inactivation which is slow but exponential with time. High concentrations of phenol would produce the other stage of inactivation at a rate which gives a linear relationship between log % survivors and log contact time. The transition in the shape of the inactivation curves with increasing phenol concentration from a linear semilog relationship to a linear log-log relationship then reflects the increasing predominance of the second stage as the phenol concentration increases. The possibility that there are two stages of inactivation by phenol is supported by the fact that phage protein consists of serologically distinguishable components, one associated with the protein coat surrounding the DNA in the "head" of the phage particle, another with the protein which constitutes the "tail" of the particle (Lanni & Lanni, 1953). Further, different proteins are precipitated by different concentrations of phenol (Cooper & Sanders, 1927, 1928). Denaturation of the phage tail protein will prevent the adsorption of the phage to the host cells (Hershey & Chase, 1952) and probably will affect the mechanism by which the phage DNA penetrates the host cell wall. The tail protein seems the more likely site of attack by low concentrations of phenol. Denaturation of the head protein may also inactivate the phage and earlier results (Cook & Brown, 1964) indicate that high concentrations of phenol also affect the association between the protein coat of the phage and its DNA.

The increase in the value of the concentration exponent for the

inactivation of the phage by phenol, when the exponent is calculated for increasing levels of inactivation, is confirmed by the previously reported values for the exponent of −14·659 calculated from mean single survival times and −15·140 from mean extinction times (Cook & Brown, 1963). This relationship is another manifestation of the effect of the concentration of phenol on the kinetics of the inactivation of the phage and it is interesting that Jordan & Jacobs (1944) found the same relationship for the inactivation of *E. coli* by phenol.

Increasing the temperature of the reaction between phage and phenol produced an increase in the rate of inactivation of the phage, but the changes in the shape of the inactivation curves corresponded to those produced by increasing the concentration of the phenol. No obvious alteration in the mechanism of inactivation therefore resulted from an increase in the temperature at which the reaction was carried out. The values of the temperature coefficient for the inactivation of the phage were independent of the level of inactivation chosen within any of the temperature ranges tested. A similar effect has been reported by Tilley (1942) and Jordan & Jacobs (1946) for the action of phenol on *E. coli*.

The temperature of incubation of phage culture plates had no effect on the number of phage particles recovering after exposure to phenol. It should be noted, however, that as the plaque counts were made using the soft agar layer method, the adsorption of the phage particles to their host took place at approximately the same temperature (46°) in all the experiments. The temperature at which adsorption takes place can affect the efficiency with which the phage infects its host (Garen & Puck, 1951) and it is at this stage that different temperatures may affect the recovery of phenol-damaged phage particles.

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