The Effect of Enzyme Inhibitors on the Multiplication of T 2 Bacteriophage

By D. E. DOLBY

Lister Institute of Preventive Medicine, Elstree, Hertfordshire

SUMMARY: Screening tests on the inhibition of growth of phage T2r⁺ and of its host, *Escherichia coli* strain B, by a number of enzyme inhibitors, showed that only arsenite, borate and cyanide inhibited the phage at concentrations which were without effect on the phage alone, on its adsorption to bacteria, or on bacterial growth. In single-step experiments, the inhibitory effect of arsenite and cyanide on phage multiplication was maintained for several hours, whereas borate merely lengthened the latent period by a few minutes without decreasing the final titre. Arsenite was still effective as an inhibitor when added half way through the latent period.

Work on the effect of inhibitors on the multiplication of bacterial viruses has been carried out almost since the discovery of bacteriophage; but it is only with the more recent interest in chemotherapy and antibiotics, and with the possibility that results with bacterial viruses might be applicable to other viruses, that it has become extensive. Compounds of many kinds have proved to inhibit virus multiplication but, with few exceptions, their mode of action is obscure. This is perhaps because the compounds were used empirically to prevent virus growth, rather than for studying the inhibitory process. The work of Cohen (Cohen, 1951; Cohen & Roth, 1953; Scott & Cohen, 1953*a, b*) on the metabolic processes of *Escherichia coli* and on the isolation of the enzymes concerned has thrown more light on the mechanisms involved in phage production; but inhibition experiments can play their part in elucidating the process. The results presented in this paper show that substances known to be enzyme inhibitors can inhibit phage production at concentrations which are without effect on bacterial growth, and that the type of inhibition may vary according to the enzymic reaction affected.

METHODS

Media. The liquid medium used (GTP broth) contained 2 % (w/v) Difco Bacto Tryptose; 0.2 % (w/v) glucose; 0.5 % (w/v) NaCl; and 0.01 M-phosphate buffer (pH 7.2).

Both phage and bacteria were diluted in a medium (phosphate saline) containing: 0.08 M-NaCl; 0.01 M-phosphate buffer (pH 7.2); 0.005 M-magnesium chloride; 0.1 % (w/v) gelatin.

Bacteriophages. *Escherichia coli* strain B, and the T series of bacteriophages were originally obtained from the late Dr W. J. Elford, F.R.S. The *E. coli* strain B, was subcultured fortnightly on nutrient agar slopes with periodical plating out to test purity of the stock, and isolation of single colonies which

were then tested to ensure that they had the same sensitivity to the T phages as the parent strain.

Concentrated bacteriophage preparations were obtained by the method of Swanstrom & Adams (1951), the final product being dialysed against M/15 phosphate buffer (pH 7.2). Titres were usually in the range $1-5 \times 10^{11}$ particles/ml. In some cases GTP broth lysates of coliphage T2r⁺ were concentrated by a modification of the method of Herriott & Barlow (1952): the lysate was mixed with acid- and alkali-washed Stellafilt (Grade SSS, Paterson Engineering Co. Ltd., 83 Kingsway, London), filtered, cooled to 0° and the pH brought to 3.9-4.0 with N-HCl. The precipitate was then centrifuged down at 0°, suspended in a small volume of saline at 0° and dissolved by addition of the minimum quantity of M-NaHCO₃. After a preliminary titration the product was diluted to a titre of 5×10^{11} with phosphate saline.

In all bacteriophage preparations sterility was maintained with a crystal of thymol.

Bacteriophage counts. These were made in duplicate by the plaque-counting method of Hershey, Kalmanson & Bronfenbrenner (1943), using digest broth solidified with 1.5% (w/v) agar for the base and with 0.75% (w/v) agar for the upper layer.

Bacterial counts. Bacterial growth was followed by viable count or by nephelometer (Evans Electroselenium Ltd.) calibrated against total counts of log phase cultures of *Escherichia coli* strain B. The viable count was always measured when growth was occurring in the presence of an inhibitor.

Inhibitor solutions. Inhibitors were usually dissolved in saline; but 8-hydroxyquinoline was dissolved in ethanol and the solution diluted with saline to below 1% (v/v) ethanol. Sulphanilamide and 2':4-dinitrophenol were dissolved in the minimum of N-NaOH, and arsenite was used either as the salt or by dissolving As₂O₃ in the minimum of N-NaOH. All solutions were brought to pH 7 before use.

SCREENING TESTS

Preliminary experiments were made with a number of substances known to inhibit a range of enzyme reactions, to discover those which would decrease phage yield at levels which were without effect on bacterial growth. An inhibitor which does not completely suppress phage multiplication may (1) increase the length of the latent period; (2) increase the length of the rise period; (3) decrease the final titre; or may produce all three effects in combination. The criterion of inhibition has usually been decrease in final titre, but changes in latent period or increase in titre may be more sensitive indicators, since partial inhibition of an enzymic process essential to phage production might decrease the rate of phage multiplication without decreasing the number of phage particles finally produced. Comparison between the titres of control suspensions of phage and bacteria and those containing inhibitors shortly after the end of the normal latent period will show if any of these types of inhibition is occurring, and also has the advantage that it does not entail the large amount of plating needed to follow growth curves. Experiment showed

that when a log-phase culture of *Escherichia coli* strain B, in GTP broth (5×10^7 organisms/ml.) was infected with an equal number of T2r⁺ particles and titrated at intervals by pipetting samples into phosphate saline at 0° to prevent further multiplication, the phage titre began to rise at 18–19 min. and had increased five- to tenfold by 20–21 min. This system was therefore made the basis of the inhibition test.

A series of tubes containing different concentrations of freshly prepared inhibitor solution (1 ml.) were brought to 37° in a water-bath and to each at 1 min. intervals added 10 ml. of a log-phase culture of *Escherichia coli* strain B, in GTP broth, containing 5×10^7 organisms/ml. as measured by nephelometer. When culture and inhibitor in each tube had been in contact exactly 10 min. 5×10^8 T2r⁺ particles in 0.1 ml. broth were added to it. After 20 min. incubation 0.1 ml. was withdrawn into 20 ml. phosphate saline at 0° to stop the phage multiplication and, after further dilution, assayed for phage. A duplicate set of tubes containing only inhibitor and bacterial culture was set up at the same time, incubated for 70–75 min. and then plated to determine the effect of the inhibitors on bacterial growth rate. Controls containing saline in place of inhibitor were included, and the initial titre of phage and viable bacteria determined on these. Experiments in which the phage multiplication in the control at 20 min., or the bacterial multiplication after 70 min., was less than fivefold were rejected. The results of a typical experiment are given in Table 1. The degree of inhibition of phage and of bacterial multiplication is expressed as an inhibition index =

$$\frac{\text{Log (final titre in presence of inhibitor/initial titre)}}{\text{Log (final titre in control/initial titre)}}.$$

Complete inhibition, or decrease of the initial titre, thus gives an index of 0; complete absence of inhibition gives an index of 1 (Table 1, cols. 4 and 6). A completely differential inhibitor of phage multiplication should decrease the inhibition index for phage below unity at a concentration that does not affect the index for *E. coli* strain B. However, substances which gave a phage index of 0.5 or less and a bacterial index of 0.9 or more have been regarded as differential inhibitors.

Twelve of the substances tested were not differential inhibitors (Table 2) and four of them were (Table 3). The compounds were tested at concentrations giving phage indexes from 0 to 1 in all cases except sodium cacodylate, which did not inhibit at 0.1 M, and sodium fluoroacetate, which did not inhibit at 100 µg./ml. (1×10^{-6} M), the highest concentration tested. With the same exceptions, all compounds were tested at concentrations giving bacterial inhibition indexes from less than 0.5 to 1. These results modify some of those presented by Czekalowski & Dolby (1949).

Effect of inhibitors on free phage and on adsorption of phage

Dilutions of phage in GTP broth containing about 5×10^7 T2r⁺ particles/ml. were incubated with each of the four active inhibitors for 4 hr. at 37° to determine whether they had any action on the phage alone (Table 4). Arsenite,

borate and cyanide were without effect at concentrations higher than those used in inhibition experiments. Proflavine inactivated coliphage T2r⁺ at 5×10^{-5} M. This is a lower inhibitory concentration than that recorded by Foster (1948), but is in accord with the results of Hedén (1951). In other experiments there was considerable destruction of coliphage T2r⁺ even at

Table 1. *Effect of various concentrations of compounds on the multiplication of bacteriophage and bacteria*

Compound	Final concentration (M $\times 10^{-3}$)	Bacteriophage		Bacteria	
		Titre after 20 min. ($\times 10^6$)	Inhibition index*	Viable count after 75 min. ($\times 10^6$)	Inhibition index*
Saline	—	297	—	353	—
Proflavine	0.001	248	0.91	366	1.02
	0.0025	143	0.63	345	0.99
	0.005	63	0.21	336	0.97
	0.01	8	0	271	0.85
	0.02	4	0	213	0.70
Zinc acetate	0.05	353	1.09	390	1.06
	0.10	365	1.11	370	1.03
	0.20	241	0.89	355	1.00
	0.50	14	0	196	0.66

Ten ml. log phase culture of *Escherichia coli* strain B in GTP broth added to duplicate tubes of 1 ml. inhibitor at -10 min. Viable count on saline control at -2 min.: 64×10^6 organisms/ml. 0.1 ml. T2r⁺ dilution added to one of each pair of tubes at 0 min. to give phage concentration: 43×10^6 particles/ml.

$$* \text{ Inhibition index} = \frac{\text{Log (final titre in presence of inhibitor/initial titre)}}{\text{Log (final titre in control/initial titre)}}.$$

5×10^{-6} M-proflavine and some evidence that destruction was less when the mixture was incubated in the dark. This apparent photo-inactivation may be responsible for the discrepancies between the results of different workers. The adsorption of phage to *Escherichia coli* strain B was not affected by the presence of arsenite (2×10^{-3} M), borate (7×10^{-3} M), cyanide (0.8×10^{-3} M), or proflavine (0.01×10^{-3} M).

THE COURSE OF PHAGE MULTIPLICATION IN THE PRESENCE OF INHIBITORS

The effects of arsenite, borate and cyanide on phage multiplication were studied in greater detail by single-step growth curves in the presence of these inhibitors. For this purpose stable suspensions of phage + bacteria were prepared in two ways:

(a) By the method of Benzer (1952), in which phage is adsorbed on to washed bacteria in a non-nutrient medium. A log phase culture in GTP broth of *Escherichia coli* strain B (1×10^8 organisms/ml.) was centrifuged at 3000 r.p.m. for 20 min., washed twice on the centrifuge with phosphate saline,

Table 2. *Inhibition indexes of compounds which did not show differential inhibition*

Compound	Final concentration (M $\times 10^{-3}$)	No. of experiments	Average inhibition index	
			Bacteriophage	Bacteria
Azide, Na	0.9	2	0	0.10
	0.6	3	0.15	0.50
	0.4	2	0.63	0.96
	0.3	4	0.90	1.03
	0.2	3	0.98	0.96
Cacodylate, Na	100	1	0.98	1.14
4:6-Dimethoxytoluquinone	2.5	1	0	0.30
	1.0	2	0	0.64
	0.5	1	1.02	0.86
	0.25	1	c. 1.6	1.08
2:4-Dinitrophenol	1.0	1	0	0.46
	0.8	2	0.5	0.73
	0.2	3	0.44	0.91
	0.15	2	1.00	0.90
Fluoride, Na	50	1	0	0.34
	25	1	0	0.61
	12.5	1	0.79	0.94
	6.2	1	1.02	1.00
Fluoroacetate, Na	0.001	3	1.05	0.96
8-Hydroxyquinoline	0.05	1	0	0.66
	0.03	2	0.28	0.83
	0.02	2	0.79	0.91
	0.015	2	0.89	1.00
	0.010	3	0.88	1.05
	0.0075	2	0.96	1.04
Monoiodoacetate, Na	0.10	1	0	0.24
	0.05	2	0.03	0.77
	0.025	2	0.61	0.93
	0.01	2	0.98	0.88
	0.005	1	1.01	0.99
Sulphanilamide	20	1	0	0.09
	15	2	0.53	0.90
	10	2	0.84	1.10
	5	1	1.00	0.96
Uranyl acetate	4.0	1	0	0
	2.0	2	0.45	0.67
	1.0	2	0.81	0.84
	0.5	2	0.99	1.04
Urethane	300	1	0	0.08
	150	1	0.19	0.80
	75	1	0.5	0.97
Zinc acetate	0.5	1	0	0.66
	0.4	1	0.14	0.72
	0.2	1	0.89	1.00
	0.1	2	1.00	1.01

resuspended in phosphate saline at about 1×10^9 organisms/ml. and aerated for 1 hr. to remove intracellular nutrients. A viable count was made and T2r⁺ particles added to a multiplicity of infection of 3–5. After adsorption at 37° for 10 min. the bacteria with adsorbed phage were centrifuged,

Table 3. *Inhibition indexes of compounds showing differential inhibition*

Compound	Final concentration (M $\times 10^{-3}$)	No. of experiments	Average inhibition index	
			Bacteriophage	Bacteria
Arsenite, K or Na	1.0	3	0	0.68 (0.66–0.72)
	0.5	3	0.38 (0.23–0.58)	0.98 (0.91–1.03)
	0.25	3	0.87 (0.73–1.08)	0.94 (0.88–0.96)
	0.10	2	1.04 (1.04)	1.01 (1.00–1.01)
Borate, Na	5.0	3	0	0.78 (0.69–0.91)
	4.0	3	0.07 (0–0.2)	0.94 (0.92–1.01)
	3.0	4	0.24 (0.09–0.33)	1.06 (1.02–1.12)
	2.0	4	0.60 (0.49–0.84)	1.08 (0.99–1.14)
	1.0	2	0.73 (0.61–0.84)	1.07 (1.05–1.08)
	0.5	1	0.83	1.16
	0.25	1	0.91	1.21
Cyanide, K	0.4	3	0	0.83 (0.78–0.85)
	0.2	3	0.30 (0.0–0.50)	0.95 (0.94–0.96)
	0.1	3	0.50 (0.41–0.68)	0.99 (0.92–1.05)
	0.05	2	0.81 (0.76–0.85)	1.00 (1.00)
Proflavine	0.01	3	0	0.90 (0.85–0.95)
	0.005	3	0.07 (0–0.21)	0.95 (0.84–1.03)
	0.0025	2	0.39 (0.15–0.63)	1.02 (0.99–1.04)
	0.001	2	0.95 (0.91–0.98)	1.01 (0.99–1.02)

Figures in parentheses show the range in individual experiments.

Table 4. *Effect of inhibitors on phage T2r⁺ alone*

Inhibitor	Titre of phage after 4 hr. at 37° ($\times 10^6$)
Saline control	44.5
Arsenite, 2×10^{-3} M	46.3
Borate, 7×10^{-3} M	41.0
Cyanide, 0.8×10^{-3} M	41.0
Proflavine, 0.05×10^{-3} M	11.1

Ten ml. phage, diluted in GTP broth, added to 1 ml. inhibitor in saline, to give the final concentration shown in Table. Incubated 4 hr. at 37°, then diluted and assayed.

washed once on the centrifuge with phosphate saline to remove free phage, and resuspended in phosphate saline at $5\text{--}10 \times 10^7$ bacteria/ml. Such suspensions, and dilutions of them in phosphate saline, were stable for at least 1 hr. at 30°, but developed normally on addition of nutrient medium and had a latent period of normal length (28 min. at 30°). In one experiment portions of a dilute suspension in phosphate saline were added 1, 15, 30 and 60 min. after preparation to GTP broth to give a count of 1.1×10^3 plaques/ml. After

100 min. incubation at 30° the respective titres were 139, 119, 146 and 134×10^3 plaques/ml. Burst sizes of over 100 were, however, unusual, the normal range being 50–100. This variation might have been due to variation in the composition of the medium and in the conditions of centrifugation and aeration. Attempts to improve reproducibility by substituting for broth the defined medium of Friedlein (1928) either alone, +0.1 % Tryptose, or + yeast extract, were unsuccessful.

(b) In later experiments a method was used which proved simpler and gave larger burst sizes. To a log phase culture of *Escherichia coli* strain B (1×10^8 organisms/ml.) in GTP broth at 37° were added cyanide or arsenite to a concentration of 1×10^{-3} M and phage T2r⁺ to give a final concentration of 1×10^5 particles/ml., and the culture was incubated for 10 min. at 37°. More than 98 % of the phage was adsorbed, as measured by the supernatant titre after centrifuging, but its development was prevented by the cyanide or arsenite. On dilution into GTP broth to bring the concentration of cyanide or arsenite below its effective level the phage developed normally and gave burst sizes over 100.

Both types of suspension were added to GTP broth at 30°, containing different concentrations of inhibitor, to give an initial count of 10^8 plaques/ml. and the increase in titre followed as usual in single step experiments. The lower temperature of incubation (30°) gave slower multiplication and more time for manipulation, the latent period being increased from 19 min. at 37° to 28 min. at 30°.

Multiplication of *Escherichia coli* strain B was followed by viable count after adding a washed aerated suspension of bacteria in phosphate saline to GTP broth + inhibitor. The initial titre was $10\text{--}30 \times 10^6$ organisms/ml. The effect of inhibitors is expressed as the ratio of the uncorrected mean generation time (MGT) of the inhibited and control cultures:

$$\frac{\text{MGT of culture + inhibitor}}{\text{MGT of control culture}}.$$

Arsenite. Fig. 1 shows that 0.25 and 0.5×10^{-3} M-arsenite had little effect on the growth of *Escherichia coli* strain B their MGT ratios being 1.08 and 1.20 respectively. A higher concentration of arsenate (1×10^{-3} M) was not inhibitory at all (MGT ratio = 1.02). But the same concentrations of arsenite greatly decreased phage yield in single step experiments (Fig. 2). The yield was never more than sevenfold in the presence of 0.25×10^{-3} M-arsenite and 0.5×10^{-3} M-arsenite suppressed multiplication completely. Arsenite thus appears a more effective inhibitor in these experiments than in the preliminary survey, in which 0.5×10^{-3} M-arsenite did not cause complete inhibition. The concentration of bacteria and phage was many thousand times higher in the early experiments and this probably explains the difference, since the number of inhibitor molecules/bacterium was higher in the single step experiments. Both methods of preparing the phage + bacteria suspensions gave similar results.

Cyanide at 0.2 and 0.4×10^{-3} M had effects similar to those of arsenite

Fig. 1. Effect of arsenite and arsenate on growth of *Escherichia coli* strain B measured by viable count. Initial titre: 18×10^8 viable organisms/ml. 37° .

Fig. 2. Effect of arsenite on growth of phage T2r⁺ in single step experiment. 30° .

(Figs. 3, 4). Doermann (1952) has already shown that at the higher concentration of cyanide (0.01M) used in his method for studying intracellular phage, there is a loss of infectious centres during the first half of the latent period but the bacteria are not lysed by the cyanide; lysis occurs only when the cyanide is added near the end of the latent period. Cyanide thus has two separate effects, inhibition of phage multiplication when it is added early in

Fig. 3. Effect of cyanide on growth of *Escherichia coli* strain B, measured by viable count. Initial titre: 24×10^8 viable organisms/ml. 37° .

Fig. 4. Effect of cyanide on growth of phage $T2r^+$ in single step experiment. 30° .

the latent period, and lysis when it is added after some intracellular phage particles are completed. Only the inhibitory effect has been studied in the present experiments.

Borate at 2 , 4 and $8 \times 10^{-3}M$ gave average MGT ratios for growth of the bacteria of 1.04, 1.08 and 1.14 respectively, with no increase in the length of the lag phase. Experiments in which the phage + bacteria suspension was prepared by washing and aeration of the bacteria gave no evidence that these concentrations of borate inhibited phage development; single step growth curves with and without inhibitor were identical. But when the suspensions

Fig. 5. Multiplication of phage T2r⁺ in single step experiment in presence of borate. Suspension of phage + bacteria, arrested by $1 \times 10^{-3}M$ -cyanide, diluted 1/100 into GTP broth + borate. 30°. ●—●, Control; ○—○, $4 \times 10^{-3}M$ -borate; ●—●, $8 \times 10^{-3}M$ -borate.

of phage + bacteria whose development had been arrested by cyanide or arsenite were diluted into GTP broth containing borate inhibition did occur. The latent period was increased from 28 min. to 30–31 min. by $2 \times 10^{-3}M$ -borate, to 31–33 min. by $4 \times 10^{-3}M$, and to 36 min. by $8 \times 10^{-3}M$, but the final titres were almost identical with that of the control, though some decrease occurred in a few experiments. When a suspension of phage + bacteria inhibited by cyanide was centrifuged down, resuspended in fresh GTP broth + cyanide, and then diluted into GTP broth + borate, the usual increase in latent period did not take place. The process of centrifugation and resuspension had evidently annulled the inhibition. This provides an explanation for the fact that suspensions of phage + bacteria prepared by washing and aeration were not inhibited by borate. The results of these experiments justify the assumption made earlier (see SCREENING TESTS) that the length of the latent period might prove a more sensitive indicator of inhibition than decrease in final titre.

Effect of time of addition of inhibitor on phage production

In experiments to determine how late in the latent period the addition of inhibitors remained effective, washed suspensions of phage + bacteria in phosphate saline were added as usual to GTP broth to start phage multiplication; further dilutions were made at intervals into broth + inhibitor at 30°, to give $1-3 \times 10^8$ plaques/ml. and the single step growth curve followed. Inhibition by arsenite occurred when the dilution into arsenite was made as late as 15 min. after the beginning of the latent period (Fig. 6), and other experiments showed complete inhibition when the dilution was made at 15 min. and partial inhibition even when it was made at 22 min. Results with cyanide were similar, although the inhibition at 10 min. was less complete and there was none at 20 min., a result to be expected from the experience of Doermann (1952) in his cyanide lysis experiments.

Fig. 6

Fig. 6. Effect of addition of 1×10^{-3} M-arsenite at different times during latent period to phage T2r+ in single step experiment. 30°.

Fig. 7

Fig. 7. Effect of removal of 0.5×10^{-3} M-arsenite at different times during latent period of phage T2r+ in single step experiment. 30°. ●—●, Control without arsenite; ○—○, arsenite 0-10 min.; ●—●, arsenite 0-20 min.; ●—●, arsenite 0-30 min.; ●—●, arsenite present throughout.

When arsenite present at the beginning of the experiment was diluted out at different times during the latent period, its effect was to delay the end of the latent period by approximately the time the inhibitor had been present (Fig. 7). In this it resembles most other phage inhibitors.

DISCUSSION

The results described in this paper show that an examination of the effect of enzyme inhibitors on phage multiplication will readily reveal some which inhibit phage differentially. Spizizen (1943) made a similar approach by testing the effect of a number of enzyme inhibitors on the multiplication of washed cells of *Escherichia coli* strain B₁ + phage P₁ in phosphate buffer + glycine anhydride (0.87×10^{-3} M) or in broth, finding complete inhibition by NaCN (2×10^{-3} M), monoiodoacetic acid (0.5×10^{-3} M), sodium arsenite (0.6×10^{-3} M), 2:4-dinitrophenol (0.5×10^{-3} M) or *p*-amino-phenol

(1×10^{-3} M). Spizizen's conditions, however, differed from those in the present work since the bacteria were unable to multiply in the glycine anhydride, and he did not test the growth of the bacteria in broth which contained the same concentration of inhibitor. The present experiments did not reveal any differential effects of moniodoacetic acid or 2:4-dinitrophenol, but did confirm those of cyanide and arsenite. The ratio concentration of inhibitor required to suppress growth of phage to concentration required to inhibit bacteria is not as great with arsenite, borate and cyanide as with some other inhibitors, but the effect was clear and reproducible.

Arsenite and cyanide inhibit respiratory and fermentative processes, arsenite by combining with SH-containing enzymes and cyanide by forming complexes with heavy metals. *Escherichia coli* strain B possesses two routes of carbohydrate breakdown, the normal fermentative one and an oxidative pathway via phosphogluconic acid and pentose phosphates. The two are in an equilibrium which may be altered by varying conditions, so that inhibition of one by cyanide or arsenite leads to increased use of the other pathway and a decrease in the effect of the inhibitor. When the cells are infected with coliphage T2r⁺, however, there is a shift in glucose metabolism from the oxidative to the anaerobic pathway (Cohen, 1951; Cohen & Roth, 1953), and the simplest explanation of the present results is that the equilibrium is disrupted by phage infection and that arsenite and cyanide inhibit the anaerobic pathway specifically.

Borate was tested for inhibitory activity because it is known to combine with ribose-5-phosphate and ribulose-5-phosphate, both products of the oxidative respiratory pathway of *Escherichia coli* (Horecker, Smyrniotis & Seegmiller, 1951). It is clear that its mode of action differs from that of cyanide and arsenite. Since the change from the oxidative to the anaerobic pathway referred to above occurs after infection with phage, borate should be effective as an inhibitor only so long as the oxidative pathway is still in use and it should not alter the phage yield. The lengthened latent period and unchanged phage yield found are thus consistent with the hypothesis that borate acts on pentose phosphate.

It is a pleasure to acknowledge a gift of sodium fluoroacetate from Professor Sir Rudolf Peters.

REFERENCES

- BENZER, S. (1952). Resistance to ultraviolet light as an index to the reproduction of bacteriophage. *J. Bact.* **63**, 59.
- COHEN, S. S. (1951). Utilization of gluconate and glucose in growing and virus-infected *Escherichia coli*. *Nature, Lond.* **168**, 746.
- COHEN, S. S. & ROTH, L. (1953). The phosphogluconate pathway of carbohydrate metabolism in the multiplication of bacterial viruses. *J. Bact.* **65**, 490.
- CZEKALOWSKI, J. W. & DOLBY, D. E. (1949). Effect of enzyme inhibitors on the genesis of phage. *Nature, Lond.* **163**, 719.
- DOERMANN, A. H. (1952). The intracellular growth of bacteriophages. I. Liberation of intracellular bacteriophage T4 by premature lysis with another phage or with cyanide. *J. gen. Physiol.* **35**, 645.

- FOSTER, R. A. C. (1948). An analysis of the action of proflavine on bacteriophage growth. *J. Bact.* **56**, 795.
- FRIEDLEIN, F. (1928). Der quantitative Verwendungsstoffwechsel des Paratyphus-B-Bazillus, des *Bacterium coli* und des *Bacillus pyocyaneus*. *Biochem. Z.* **194**, 273.
- HEDÉN, C.-G. (1951). Studies of the infection of *E. coli* B with the bacteriophage T2. *Acta path. microbiol. scand. Supplementum* **88**.
- HERRIOTT, R. M. & BARLOW, J. L. (1952). Preparation, purification and properties of *E. coli* virus T2. *J. gen. Physiol.* **36**, 17.
- HERSHEY, A. D., KALMANSON, G. M. & BRONFENBRENNER, J. (1948). Quantitative methods in the study of the phage-antiphage reaction. *J. Immunol.* **46**, 267.
- HORECKER, B. L., SMYRNIOTIS, P. Z. & SEEGMILLER, J. E. (1951). The enzymatic conversion of 6-phosphogluconate to ribulose-5-phosphate and ribose-5-phosphate. *J. biol. Chem.* **193**, 383.
- SCOTT, D. B. MACN. & COHEN, S. S. (1953*a*). The oxidative pathway of carbohydrate metabolism in *Escherichia coli*. 1. The isolation and properties of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. *Biochem. J.* **55**, 23.
- SCOTT, D. B. MACN. & COHEN, S. S. (1953*b*). The oxidative pathway of carbohydrate metabolism in *Escherichia coli*. 2. Quantitative studies of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. *Biochem. J.* **55**, 33.
- SPIZIZEN, J. (1943). Biochemical studies on the phenomenon of virus reproduction. II. Studies on the influence of compounds of metabolic significance on the multiplication of bacteriophage. *J. infect. Dis.* **73**, 222.
- SWANSTROM, M. & ADAMS, M. H. (1951). Agar layer method for production of high titre phage stocks. *Proc. Soc. exp. Biol., N.Y.* **78**, 372.

(Received 7 October 1954)