THE EFFECT OF 2,4-DINITROPHENOL AND PHAGE T2 ON ESCHERICHIA COLI B

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Monod (1944) has shown that in suitable concentrations the metabolic inhibitor 2,4-dinitrophenol (DNP) prevents formation of both constitutive and adaptive enzymes in *Escherichia coli* while permitting the enzymes already present to function. Growth is inhibited and respiration continues at a constant rate. These properties of the inhibitor have been confirmed with the strain of *E. coli* B used for these studies. If DNP is added to a culture, the rate of oxygen uptake will be constant and will represent the respiratory enzyme activity at the moment when DNP was added. This is very useful for experiments on adaptive enzymes. However, during experiments on adaptive enzymes in cultures infected with bacteriophage T2r$^+$ it was found that when DNP was added the respiratory activity decreased and lysis of the infected cultures was accelerated (Heagy, 1949). The effects of inhibition by DNP and infection with phage T2r$^+$ on cultures of *E. coli* B will be described in this paper.

MATERIALS AND METHODS

Bacteria. Stock cultures of *Escherichia coli*, strain B, are maintained on agar slopes at refrigerator temperature and subcultured monthly. For experiments the fluid medium used was inoculated 1:100 from a 24-hour culture grown in the same medium. After 3 to 4 hours the culture was centrifuged for 10 minutes, the supernatant was discarded, and the cells were resuspended in fresh medium. After dilution to suitable turbidity, the washed suspension was added to the experimental tubes.

Bacteriophage. With the exception of a few experiments with phage T1, all the experiments were done with either phage T2r$^+$ or phage T2r. The usual bacteriophage: bacteria ratio was about 3:1 to assure that most of the bacteria would be infected.[2]

Synthetic medium. Medium S_2 of Monod and Wollman (1947), of the following composition, was used: KH_2PO_4, 1.5 g; $Na_2HPO_4 \cdot 12H_2O$, 16.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NH_4Cl, 2.0 g; $CaCl_2$, 0.01 g; $FeSO_4 \cdot 7H_2O$, 0.0005 g; redistilled water, 1,000 ml; pH, 7.5.

Nutrient broth. Difco nutrient broth, dehydrated, 8 g per liter, pH 6.8.

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[2] *E. coli* B and phage T1 were kindly supplied by Dr. M. Delbrück, California Institute of Technology. Phages T2r and T2r$^+$ were kindly supplied by Dr. I. N. Asheshov, New York Botanical Garden.

Agar plates. Papain digest medium (Asheshov, 1941) with 0.9 per cent agar and 0.25 per cent Difco yeast extract. Thirty ml poured in 90-mm petri dishes. Plates dried in incubator for 1 hour before use.

2,4-Dinitrophenol. Stock solution of 5.4×10^{-2} M 2,4-dinitrophenol (B.D.H.) in medium S_2. Used in final concentration of 2.3×10^{-3} M unless otherwise noted.

Glucose or lactose. Analar grade. Stock solutions of 10 per cent sugar in synthetic medium were sterilized by filtration and were used in final concentration of 0.2 per cent unless otherwise noted.

Bacterial and phage counts. Bacterial colony counts and bacteriophage clearing counts were made by the method of Asheshov *et al.* (1933).

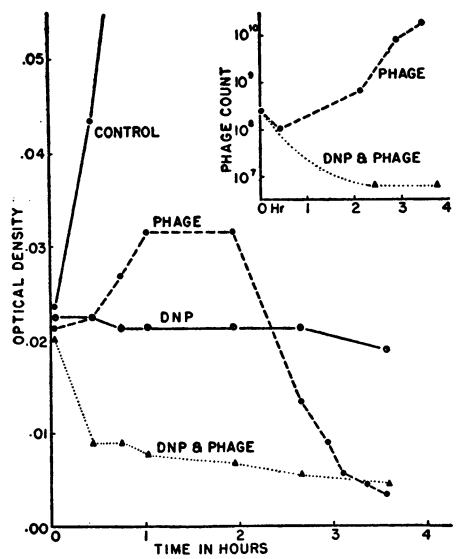

Figure 1. Effect of 2.3×10^{-3} M DNP on *E. coli* infected with phage T2r$^+$ in nutrient broth; 1.3×10^8 bacteria per ml and 2.6×10^9 phage particles per ml added.

Turbidimetric experiments. Cultures were grown in Evelyn colorimeter tubes and readings were taken in an Evelyn photoelectric colorimeter with filter no. 660. Between readings the tubes were shaken in a water bath at 37 C.

RESULTS

Lysis of infected cells with dinitrophenol. When a culture in nutrient broth was simultaneously infected with phage T2r$^+$ and inhibited with DNP, lysis began almost immediately, as shown in figure 1. Phage alone did not cause lysis till after a latent period, the length of which depended on the extent of "lysis inhibition" caused by the r$^+$ factor, as described by Doermann (1948). Usually lysis was delayed for 1.5 hours or longer. DNP inhibited growth of uninfected bacteria and did not cause massive lysis of the culture, although in many experiments there was a small decrease in turbidity. Normal phage lysis was accompanied by the release of daughter phage particles, but lysis by the combined

actions of phage and DNP was not accompanied by the production of more phage, and even the phage used to infect the culture was lost.

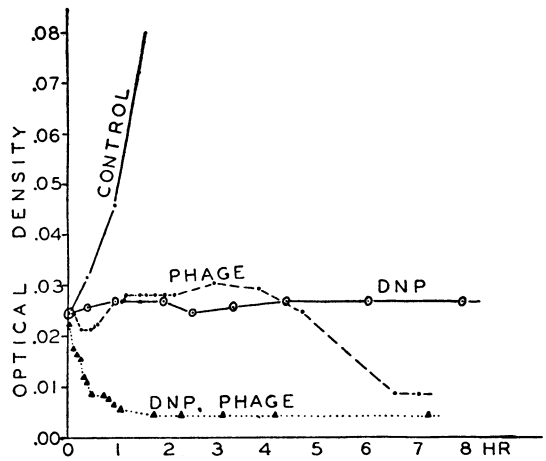

Figure 2. Effect of 2.3×10^{-3} M DNP on *E. coli* infected with phage T2r$^+$ in synthetic medium with 0.2 per cent glucose; 1.1×10^8 bacteria per ml and 4.3×10^8 phage particles per ml added. Final phage counts: "Phage"—1.3×10^9 per ml; "DNP and Phage"—1.2×10^8 per ml.

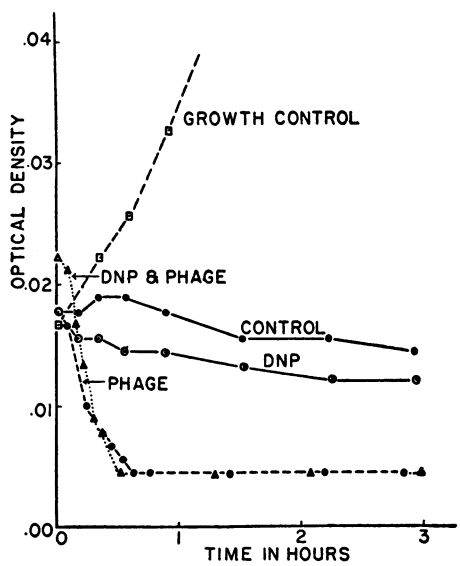

Figure 3. Effect of 2.3×10^{-3} M DNP on *E. coli* infected with phage T2r$^+$ in synthetic medium without carbohydrate source; 1.0×10^8 bacteria per ml and 6.0×10^8 phage particles per ml added. "Growth control" had 0.2 per cent glucose.

The same phenomena were observed when synthetic medium with 0.2 per cent glucose was used for growing the bacteria and for the actual experiment (figure 2). But if the bacteria were grown with 0.2 per cent glucose and washed and resuspended in synthetic medium without carbohydrate, infection with

phage T2r$^+$ produced immediate lysis of the culture whether DNP was present or not (figure 3). A similar result was observed when bacteria that had been grown with 0.2 per cent glucose were infected with phage T2r$^+$ in synthetic medium containing only 0.2 per cent lactose as the source of carbohydrate (figure 4). In 1 to 1.5 hours the control adapted to lactose as a source of carbohydrate.

Effect of DNP on phage T2r$^+$ alone. To test the effect of DNP on phage in the absence of bacteria a tube containing 2×10^8 phage T2r$^+$ per ml in synthetic medium was compared with a tube containing the same concentration of phage in the same medium with 2.3×10^{-3} M DNP. The tubes were incubated with shaking at 37 C for 2 hours and then left standing at room temperature for 50 days. Estimations of phage activity were made at intervals, and no change in

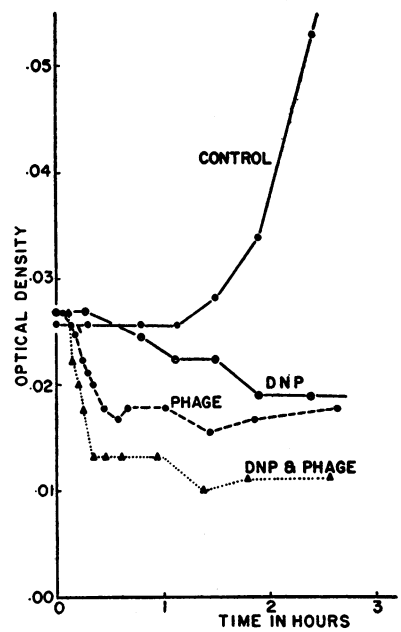

Figure 4. Effect of 2.3×10^{-3} M DNP on *E. coli* infected with phage T2r$^+$ in synthetic medium with 0.2 per cent lactose as carbohydrate source (bacteria not adapted to lactose); 1.0×10^8 bacteria per ml and 4.0×10^8 phage particles per ml added. Final phage counts: "Phage"—2.7×10^7 per ml; "DNP and Phage"—2.6×10^7 per ml.

phage concentration occurred in either the tube with DNP or the control tube.

On another occasion the effect of 2.3×10^{-3} M DNP was tested on both phage T2r$^+$ and phage T2r in a low concentration in (a) nutrient broth, (b) synthetic medium, and (c) synthetic medium with 0.2 per cent glucose (table 1). In no case was there any difference due to the presence of DNP. An interesting feature of this experiment was the increased loss of phage in the presence of 0.2 per cent glucose. This may be related to Delbrück and Luria's observation (Delbrück, 1946) that growth of phage T2 is very susceptible to products developed from glucose under some conditions of preparation of the medium, and that phages T1 and T5 are rapidly inactivated in a high dilution in pure medium.

Action of DNP on cells infected with phage T2r or phage T1. DNP completely

inhibited lysis of cells infected with phage T2r both in nutrient broth and in synthetic medium with 0.2 per cent glucose (figure 5). Phage T1 has also been used, and DNP completely inhibited the lysis of cells infected with it.

TABLE 1

Phage counts after incubation at 37 C with 2.3 × 10^{-3} M DNP in various media*

PHAGE†	DURATION OF INCUBATION	NUTRIENT BROTH		SYNTHETIC MEDIUM			
				No carbohydrate		0.2% glucose	
		No DNP	DNP	No DNP	DNP	No DNP	DNP
	hr						
T2r+	0	74	77	83	77	79	76
	24	46	80	30	28	2	1
	96	32	55	19	11	0.0	0.0
T2r	0	47	64	59	56	62	45
	24	49	42	11	20	4	2
	96	42	34	5	7	0.0	0.0

* Multiply by 3.2 × 10^4 for phage per ml.

† These phages were grown on *E. coli* in synthetic medium with 0.2 per cent glucose. A similar set of tubes with phages grown on cells in nutrient broth gave similar results.

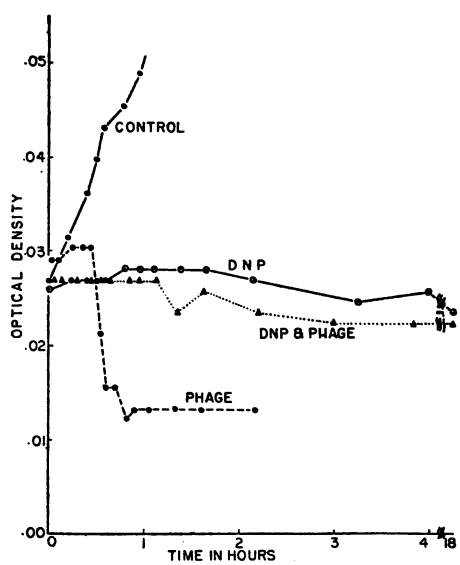

Figure 5. Effect of 2.3 × 10^{-3} M DNP on *E. coli* infected with phage T2r in synthetic medium with 0.2 per cent glucose as carbohydrate source; 1.0 × 10^8 bacteria per ml and 1.4 × 10^8 phage particles per ml added. Final phage counts: "Phage"—1.6 × 10^9 per ml; "DNP and Phage"—0.7 × 10^8 per ml (2.5 hr), 1.1 × 10^6 per ml (18 hr).

DISCUSSION

In nutrient broth or in synthetic medium there is almost immediate lysis of cultures of *E. coli* that are simultaneously infected with phage T2r+ and

exposed to DNP. Phage formation is prevented and the infecting phage is lost. These effects seem to be analogous to results reported by Cohen (1949) in a recent review. He found that "when the oxidative respiration of strains of *E. coli* is inhibited by depleting the medium of oxygen with a stream of nitrogen, or by the addition of cyanide or iodoacetate to the medium, infection with phage T2r$^+$ induced lysis from the moment of infection." Phage was not synthesized and the infecting phage was lost. Cohen offers the explanation that infection with T2 may produce "a rapid reorganization of cell substance, which in the absence of the host's energy supply is uncontrollable, leading to autolysis and disrupting of the structures essential to phage synthesis." Cohen states that there is marked lysis of cyanide-treated cells upon the addition of phage T2r$^+$ and T4r$^+$, but lysis does not occur with T7 and T4r—the observation with T4r having been made by Doermann.

Although DNP does not inhibit respiration, it interferes with the coupling between oxidation and phosphate uptake (Hotchkiss, 1944). Loomis and Lipmann (1948) made the interesting observation that DNP can "replace" inorganic phosphate during the oxidation of glutamate by rabbit kidney homogenate, indicating that DNP reversibly uncouples phosphorylation from oxidation. This observation has been confirmed by Cross *et al.* (1949). Thus, although it does not inhibit respiration, DNP resembles the inhibitors used by Cohen in that it interferes with the energy metabolism of the cell. If the effects of DNP on phage-infected cultures are analogous to those of the inhibitors used by Cohen, then phages T2r and T1 resemble phages T7 and T4r in not being associated with lysis when infected cultures are exposed to these agents.

Although normal cultures in media with utilizable carbohydrate did not lyse in the presence of DNP, there was partial lysis of cultures that lacked utilizable carbohydrate, i.e., cells that were in medium without carbohydrate, or unadapted cells that were transferred to medium with lactose as the carbohydrate source and prevented from adapting to it by the DNP. This may be related to the observation made by Monod (1942) that sometimes there was marked lysis of cultures of *Bacillus subtilis* when the carbohydrate substrate was exhausted. Apparently the nature of the carbohydrate with which the cells had been grown determined whether or not they would lyse when deprived of a source of carbohydrate.

Cultures that lacked utilizable carbohydrate showed immediate lysis when infected with phage T2r$^+$ even though no DNP was present. Monod and Wollman (1947) observed a gradual decrease of the turbidity of cultures of *E. coli* B infected with a phage (not belonging to the "T" system) in medium containing no utilizable carbohydrate. The difference in the rate of lysis in their experiments and those reported here may be due to the different phages used.

Interference with the energy metabolism of the bacterial cell is the one feature common to all the agents that have been used in these experiments—phage infection, lack of available carbohydrate, inhibition by DNP, and, in Cohen's experiments, inhibition by anaerobiosis, cyanide, or iodoacetate. Experiments are in progress to investigate the mechanism of lysis when cells are infected

with phage and exposed to DNP, but no speculation will be made about it at this time. The picture is confusing. The r^+, or lysis-inhibiting, phage inhibits lysis when it reinfects an already infected bacterium, but the rapid-lysing (r) phage is not able to do this (Doermann, 1948). But in the presence of inhibition by DNP it has been found that lysis by the lysis-inhibiting (r^+) phage is accelerated, although lysis by the rapid-lysing (r) phage is inhibited!

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SUMMARY

Simultaneous infection with phage T2r^+ and inhibition by 2,4-dinitrophenol is followed by immediate lysis of *Escherichia coli* B in nutrient broth or in a synthetic medium containing ammonia, salts, and glucose or lactose, or no carbohydrate. The infecting phage is lost and no new phage is produced.

Dinitrophenol inhibits lysis of *E. coli* B infected with phage T2r or with phage T1.

In synthetic medium without carbohydrate, infection with phage T2r^+ is followed by immediate lysis even in the absence of dinitrophenol.

In synthetic medium without carbohydrate, exposure of an uninfected culture of *E. coli* B to dinitrophenol is followed by partial lysis.

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