citrate was the substrate, no protection by pyruvate was found; in this case the enzymes related to citrate synthesis were not involved, and the inhibition would depend only on the block of aconitase.

These results indicate that glyoxylate acts in two different ways, both connected with the control of the rate of the citric acid cycle. The first involves the inhibition of aconitase by oxalomalate produced by condensation with oxaloacetate; the second may be a possible competition with acetate at the level of the enzyme system related to citrate synthesis.

SUMMARY

1. The effect of the addition of small amounts of glyoxylate (with or without oxaloacetate), and of oxalomalate, to respiring rat-liver mitochondria and to rabbit-kidney cyclophorase preparations was investigated under different conditions.

2. Glyoxylate plus oxaloacetate strongly inhibited the oxidation of L-glutamate, oxoglutarate or pyruvate in intact mitochondria, without a parallel decrease of the P:O ratio.

3. When oxidative phosphorylation was uncoupled, either by the addition of 2,4-dinitrophenol to intact mitochondria and cyclophorase preparations, or by using damaged mitochondria, oxygen uptake with L-glutamate and pyruvate as substrates was still inhibited by glyoxylate plus oxaloacetate.

4. Glyoxylate alone also inhibited the oxidation of pyruvate, citrate, oxoglutarate and succinate in intact mitochondria. When the substrates were pyruvate or citrate the inhibition was accompanied by a consistent accumulation of citrate, but no accumulation was found when the substrate was succinate or oxoglutarate. When oxaloacetate was added in addition to glyoxylate, the accumulation of citrate increased considerably with pyruvate or citrate as substrate but only slightly with oxoglutarate or succinate.

5. Oxalomalate inhibited the oxidation of all the substrates tested, but, compared with that of glyoxylate, the inhibition was higher on citrate or pyruvate than on oxoglutarate or succinate. Glyoxylate behaved in the opposite way.

6. The inhibition by glyoxylate of the oxidation of oxoglutarate and of succinate, but not of that of citrate, was abolished by an equimolecular concentration of pyruvate.

7. The possibility that glyoxylate, besides inhibiting aconitase through the formation of oxalomalate, may interfere with some other enzymic system of the cycle is discussed.

REFERENCES

Ettinger, R. H., Goldbaum, L. R. & Smith, L. H. (1952). *J. biol. Chem.* **199**, 531.

Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.

Gaull, G. & Villee, C. A. (1960). *Biochim. biophys. Acta*, **39**, 560.

Green, D. E., Loomis, W. F. & Auerbach, V. H. (1948). *J. biol. Chem.* **172**, 389.

Judah, J. D. (1951). *Biochem. J.* **49**, 271.

Kalnitsky, G. & Tapley, D. F. (1958). *Biochem. J.* **70**, 28.

Kunitz, M. & McDonald, M. R. (1948). *J. gen. Physiol.* **27**, 393.

Lardy, H. A. & Wellman, H. (1952). *J. biol. Chem.* **195**, 215.

Ruffo, A., Romano, M. & Adinolfi, A. (1959). *Biochem. J.* **72**, 613.

Ruffo, A., Testa, E., Adinolfi, A. & Pelizza, G. (1962). *Biochem. J.* **85**, 588.

Schneider, W. C. (1949). In *Manometric Techniques and Tissue Metabolism*, p. 153. Ed. by Umbreit, W. W., Burris, R. H. & Stauffer, J. F. Minneapolis: Burgess Publishing Co.

Singer, T. P., Kearney, E. B. & Massey, V. (1957). *Advanc. Enzymol.* **18**, 65.

Tyler, D. B. (1960). *Biochem. J.* **77**, 293.

Biochem. J. (1962) **85**, 600

Studies on the Deoxyribonucleases of Bacteriophage-Infected *Escherichia coli*

BY A. B. STONE* AND K. BURTON

Medical Research Council Cell Metabolism Research Unit, Department of Biochemistry, University of Oxford

(*Received* 24 *July* 1962)

Extracts prepared from *Escherichia coli* after infection with bacteriophages T2, T4, T5 or T6 show higher deoxyribonuclease activities than do comparable extracts from uninfected bacteria (Pardee & Williams, 1952, 1953; Kozloff, 1953; Kunkee & Pardee, 1956; Crawford, 1959). Kozloff (1953) suggested that the increased activity is due to the phage causing the destruction of a minor

* Present address: Division of Biology, California Institute of Technology, Pasadena, Calif., U.S.A.

RNA component which normally inhibits the bacterial deoxyribonuclease. Our observations (Stone & Burton, 1961), described in full in this paper, do not support this view and instead suggest that the higher deoxyribonuclease activities in phage-infected bacteria are the result of the synthesis of one or more enzymes after infection.

MATERIALS AND METHODS

Deoxyribonucleic acid. DNA was obtained by deproteinizing herring-testes nucleoprotein (Daly, Allfrey & Mirsky, 1949) according to the method of Emanuel & Chaikoff (1953). The material was precipitated by the addition of ethanol, dialysed against mM-NaCl and reprecipitated by the addition of ethanol. Solutions (0·2%) were prepared in 10 mM-NaCl. For heat denaturation, they were heated at 100° for 30 min. and rapidly cooled. This treatment increased the extinction at 260 mμ by 36%.

Ribonucleic acid. RNA was obtained from a stationary-phase culture of *E. coli* B in salts–mannitol medium. The cells were washed with 0·14 M-NaCl and crushed in a bacterial press (Hughes, 1951) at −20°. The material was homogenized with 1 vol. of 90% (w/w) phenol and centrifuged. The phenol layer was extracted with three successive portions of water and these were added to the original aqueous layer (cf. Kirby, 1956). Phenol was removed from this solution by ether extraction. Contaminating DNA was degraded with pancreatic deoxyribonuclease. The enzyme was inactivated by heating for 20 min. at 100° and shaking with chloroform. Denatured protein was removed by centrifuging and the RNA was precipitated by the addition of ethanol, dried, dissolved in 10 mM-NaCl and stored at −20°.

Buffers. Tris buffer solutions were prepared by mixing N-HCl and M-tris to obtain the indicated pH; glycine buffers were similarly prepared from N-NaOH and M-glycine, and phosphate buffers from M-KH_2PO_4 and M-Na_2PO_4. The concentrations indicated in the experimental details are the final concentrations of tris, glycine or phosphate. All pH measurements were made at room temperature with a glass electrode.

Deoxyribonuclease from Escherichia coli. *E. coli* B (8 g. wet wt.) was crushed in a bacterial press (Hughes, 1951), suspended in 200 ml. of 0·2 M-tris buffer, pH 7·4, and incubated at 37° for 7 hr., during which the RNA was enzymically degraded. The DNA was then degraded by adding 30 mM-$MgSO_4$ and incubating for a further 4 hr. The solution was clarified by centrifuging and dialysed at 20° first against 2 M-NaCl and then against water. The preparation contained 1·8 mg. of protein/ml. and had an activity (see below) of 33 μm-moles/min./ml. with native DNA at pH 7·2 and optimum concentrations of $MgCl_2$ (20–50 mM).

Bacteriophages. Stocks of T2r, T2r$^+$ and T6r$^+$ phages were grown and purified by the methods used by Burton (1955). For the preparation of T5, *E. coli* B/2 was grown at 37° in 5 l. of aerated medium which contained tryptone (Oxo Ltd.) (0·7%), NaCl (0·45%), glucose (0·02%) and $CaCl_2$ (5 mM). After about 2 hr., when the turbidity was at a minimum, debris was removed by passing the suspension at 100 ml./min. through a Sharples centrifuge running at 12 000 rev./min. The supernatant was then centrifuged in a Servall SS-3 continuous-flow centrifuge at a flow rate of 8–10 ml./min. The pellets were suspended by gentle dispersion in a hand-operated glass homogenizer in 100 ml. of a solution which contained KH_2PO_4 (0·2%), Na_2HPO_4 (0·3%) and $MgSO_4,7H_2O$ (0·04%). The suspension was centrifuged at 2000*g* for 15 min. and the supernatant was incubated at 37° for 20 min. with 1 mg. of pancreatic deoxyribonuclease in the presence of $MgCl_2$ (20 mM) and $CaCl_2$ (2 mM). The preparation was purified by three cycles of differential centrifuging. About 10^{14} particles were obtained.

Bacteriophages were assayed by the double-layer technique (Adams, 1950) with *E. coli* strain B or, for T5, strain B/2 (see below). Strain B/2 is similar to strain F (Lanni, 1958) in giving a high plating efficiency for T5.

Other materials. Chloramphenicol was supplied by Parke, Davis and Co. Crystalline ribonuclease and deoxyribonuclease from bovine pancreas were from Sigma Chemical Co. (St Louis 18, Mo., U.S.A.); no deoxyribonuclease activity was detected in the ribonuclease.

Growth of bacteria and infection with phages. Bacteria were grown at 37° with aeration. A broth medium (Putnam, Kozloff & Niel, 1949) was used for the experiment of Fig. 2; otherwise the medium consisted of (amounts/l.): 3·4 g. of Na_2HPO_4, 1·5 g. of KH_2PO_4, 1·0 g. of NH_4Cl, 6·0 g. of NaCl, 10 mg. of $MgSO_4$, 0·16 mg. of $FeCl_3$ and 10 g. of mannitol. A 12 hr. culture was added to 10 vol. of fresh medium and, when the concentration of bacteria reached about 5×10^8/ml. (2–2½ hr.), they were infected by the addition of 5–7 infective phage particles/cell. For phage T5, $CaCl_2$ (final concn. 1 mM) was added to the culture just before infection. The bacteria were *E. coli.* strain B or, in some of the experiments with phage T5, strain B/2, which is a mutant of B resistant to phage T2. The B/2 strain used was originally isolated by Dr S. E. Luria.

Preparation of cell-free extracts. Portions (30 ml.) of culture were mixed with about 15 g. of crushed ice, and the bacteria were collected by centrifuging. They were suspended in 4 ml. of water in a 2·5 cm. diam. glass tube which was cooled in an ice bath. The suspension was treated for 4 min. with vibrations from a Mullard–MSE ultrasonic disintegrator, working at 19 kcyc./sec. and 60 w, with a 1·9 cm. diam. stainless-steel parallel probe (Hughes, 1961). For some experiments (Figs. 4 and 7), 70 ml. portions of culture were taken, the cells were suspended in 8 ml. of 0·01 M-phosphate buffer, pH 7·3, and a 58/20 mm. titanium probe was used for 3 min. The resulting extracts were clear and almost colourless. In each series of extracts from an infected culture, there were, within ±5%, the same amounts of protein (1–2 mg./ml.).

Deoxyribonuclease activity. The compositions of the incubation mixtures are given for the individual experiments. After incubation at 37° for the times given, the reactions were stopped by the addition of 0·1 vol. of 2·5 N-perchloric acid. After 30 min. at 4°, the mixtures were centrifuged and deoxyribose was determined in the supernatants according to the methods of either Dische (1930) or Burton (1956). (Pyrimidine derivatives of deoxyribose do not react in these methods.) Corrections were applied for unincubated controls which contained 2–10 μM-deoxyribose and for the effects of tris, glycine and $MgCl_2$ which caused small reductions (less than 10%) in the colour obtained. Deoxyribose was used as the standard.

The enzyme activity is expressed as μm-moles of acid-soluble purine-bound deoxyribose liberated/min. With untreated or ribonuclease-treated extracts of either normal or phage-infected cells, the amount of acid-soluble deoxyribose formed was proportional both to the time of incubation (up to 3 hr.) and to the amount of extract. The duration (4–15 min.) of ultrasonic treatment, and the amount of pancreatic ribonuclease (10–50 μg.), were not critical.

Phosphorylation of thymidylate. The activity of the enzyme system was determined from the formation of thymidine triphosphate (Fessler, Kelemen & Burton, 1960).

Analytical methods. RNA ribose was determined by the orcinol reaction (Mejbaum, 1939) with a reagent containing 0·33 g. of $FeCl_3$/l. (Ogur & Rosen, 1950) and a heating time of 45 min. Ribose was used as the standard and corrections were applied for the slight reaction of any DNA. DNA was determined in bacterial cultures as described by Burton (1956). Since pyrimidine-bound ribose and deoxyribose do not react in these methods, the values obtained were arbitrarily multiplied by 2. Protein was determined according to Lowry, Rosebrough, Farr & Randall (1951), with dried crystalline bovine serum albumin (Armour Laboratories, Eastbourne) as the standard. Extinction measurements were made in a Beckman model DU spectrophotometer with 1 cm. quartz cells.

RESULTS

During the first 15 min. after infection the deoxyribonuclease activity in extracts from T2r- infected cells increased to almost three times the activity of extracts from uninfected bacteria (Fig. 1). When the extracts were incubated with pancreatic ribonuclease, the endogenous RNA was degraded to acid-soluble fragments and the deoxyribonuclease activities were increased a further three- to four-fold. There was no appreciable change of deoxyribonuclease activity on incubation for the same time in the absence of pancreatic ribonuclease. The phage-induced increase of deoxyribonuclease activity found in the ribonuclease-treated extracts was at least as great as that in the untreated extracts. Similar results were obtained with phage T6r$^+$ (Fig. 2) under conditions which were closely comparable to those of Kozloff (1953) in that the same phage was used and the medium was of the same composition.

Phage T5 gave a similar result to that shown for phage T2 except that the increase of deoxyribonuclease activity occurred between 10 and 25 min. after the infection (cf. Fig. 7).

Optimum conditions for deoxyribonuclease activity. Extracts from normal bacteria and from bacteria 60 min. after infection with T5 were treated with ribonuclease to destroy endogenous inhibitory RNA, and their deoxyribonuclease activities were estimated on either native or heat-denatured DNA at pH 7 and at pH 8·5 (Table 1). Higher deoxyribonuclease activities were always obtained from the infected cells. The increase due to infection was greatest with the heated DNA at pH 8·5. Similar results were obtained for phage T2r$^+$. For both phages, the differences between the deoxyribonuclease activities of the uninfected and infected

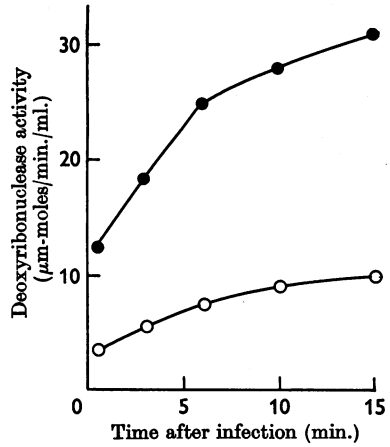

Fig. 1. Deoxyribonuclease activities in extracts from T2r- infected *Escherichia coli* B. Ultrasonic extracts (about 1 mg. of protein/ml.) were prepared at the times indicated (the 0 min. sample was uninfected). Extract (0·3 ml.), phosphate buffer, pH 7·4 (14 mM), $MgSO_4$ (36 mM) and DNA (1 mg.) were incubated in a volume of 1·4 ml. for 2 hr. For treatment with ribonuclease, the above amounts of extract and buffer were incubated with 10 μg. of pancreatic ribonuclease in a volume of 0·6 ml. for 1 hr. before the addition of DNA and $MgSO_4$. They were then incubated for a further 2 hr. ○, Extract not treated with ribonuclease; ●, extract treated with ribonuclease.

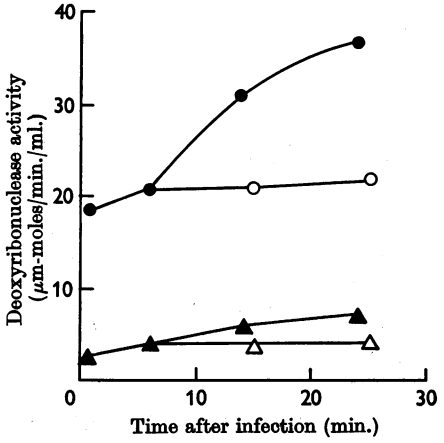

Fig. 2. Effect of chloramphenicol on the increase in deoxyribonuclease activity after the infection of a broth culture of *Escherichia coli* B with T6r$^+$. The procedure was as described in Fig. 1. ▲, △, Before ribonuclease treatment; ●, ○, after ribonuclease treatment. Closed symbols: no chloramphenicol added; open symbols: chloramphenicol (20 μg./ml.) added to the culture 5 min. after infection.

Table 1. *Effect of* pH *and form of substrate on deoxyribonuclease activities of normal and T5-infected* Escherichia coli

Extracts were prepared from *E. coli* B/2 or from part of the same culture 32 min. after infection with T5. The extracts, containing about 1·6 mg. of protein/ml., were mixed with 0·05 vol. of ribonuclease (1·2 mg./ml.) in 0·5M-tris buffer, pH 8·0. After 20 min. at 37°, 0·3 ml. portions were added to 0·8 ml. portions of a mixture containing buffer (glycine, pH 9·0, or tris, pH 7·1) (0·1M), DNA (0·7 mg.), $MgCl_2$ (25 mM) and NaCl (5 mM). The release of acid-soluble deoxyribose was measured after 20 and 40 min. at 37°.

		Deoxyribonuclease activity (μm-moles/min./mg. of protein)			
DNA	pH	Not infected	T5-infected	Increase on infection	Percentage increase
Native	7·0	10·7	17·8	7·1	67
Heated	7·0	5·4	12·7	7·3	135
Native	8·5	14·5	33·9	19·4	134
Heated	8·5	7·8	32·2	24·4	315

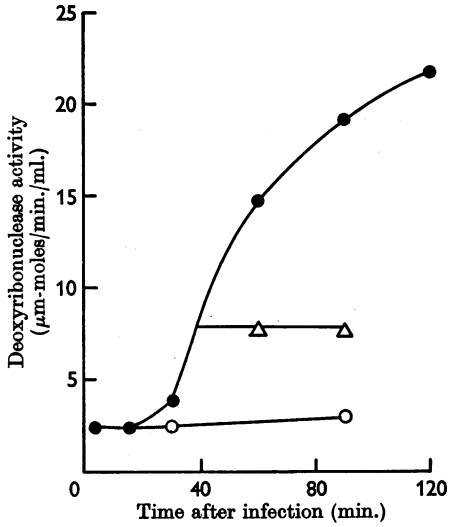

Fig. 3. Effect of chloramphenicol on the increase in deoxyribonuclease activity after the infection of *Escherichia coli* B/2 with T5. The procedure was as described in Table 1. The extracts had been treated with ribonuclease and activities were measured on heat-denatured DNA at pH 8·8. Chloramphenicol (50 μg./ml.) was added to the culture medium at 5 min. (○) or 40 min. (△) after infection; ●, no chloramphenicol added.

extracts were greatest at about pH 9. Negligible activity was detected in the absence of added Mg^{2+} ions. When the activity was measured with heated DNA and with pH 9·2 buffer, the activity of ribonuclease-treated extracts from bacteria infected with T5 rose, in some experiments, to nine times that before infection (e.g. Fig. 3).

Effect of chloramphenicol. The experiments of Figs. 2 and 3 also show that the addition of chloramphenicol prevented subsequent increase in deoxyribonuclease activities, suggesting that the increase required concomitant synthesis of protein.

Time of treatment with ribonuclease. Fig. 4 shows the deoxyribonuclease activities of extracts from infected and uninfected bacteria after treatment of the extracts with ribonuclease for different periods. The higher activities obtained from infected cells are evident after all periods of ribonuclease treatment which were studied.

Inhibition of Escherichia coli *deoxyribonuclease by* Escherichia coli *ribonucleic acid.* As is shown in Fig. 5, phenol-extracted *E. coli* RNA inhibited the activity of a crude preparation of *E. coli* deoxyribonuclease by 50 % at about 0·1 μmole of RNA ribose/ml. and 80 % at 0·5–2·0 μmoles/ml. There was no inhibition when the RNA had been treated with pancreatic ribonuclease.

Because of large differences in the concentrations of substrate and inhibitor, our results are not quantitatively comparable with those of Lehman, Roussos & Pratt (1962) for the competitive inhibition by RNA of a purified deoxyribonuclease from *E. coli*.

To determine if phage infection destroys part or all of the inhibitory RNA, ultrasonic extracts from normal and phage-infected bacteria were heated at 100° to denature endogenous nucleases and their inhibitory effects were examined. As is shown in Table 2, there was no significant difference between the inhibitions caused by the various extracts. There was no inhibition when the heated extracts had been treated with pancreatic ribonuclease.

Effects of p-*chloromercuribenzoate.* The deoxyribonuclease activity which appears after infection by phage T2 is more sensitive to inhibition by 0·03 mM-*p*-chloromercuribenzoate than are the activities in uninfected or T5-infected bacteria (Fig. 6).

Time of synthesis of deoxyribonuclease. The above experiments suggest that the increase in activity is due to the synthesis of deoxyribonuclease after infection. The enzyme appears at about the same time as the formation of the enzymes concerned in the biosynthesis of phage DNA. The time of syn-

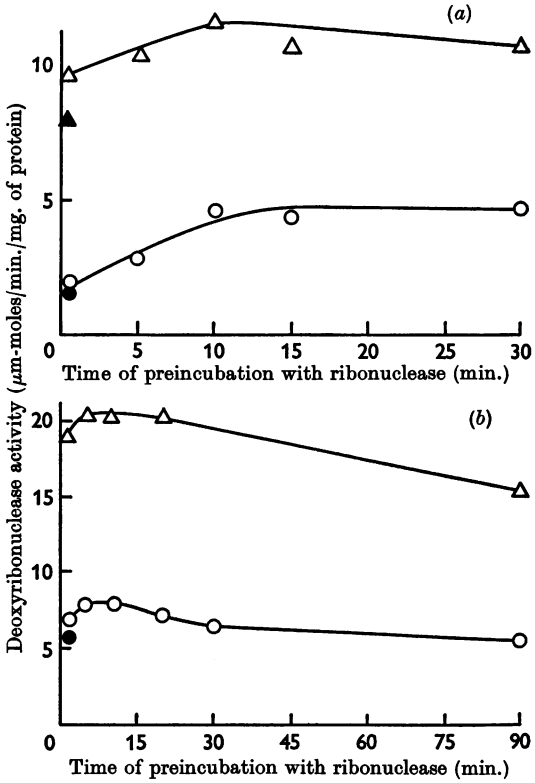

Fig. 4. Effect of incubation with ribonuclease for different times before measurement of deoxyribonuclease activity. To extracts from infected or uninfected *E. coli* B was added 0·05 vol. of ribonuclease (1·2 mg./ml.) in 0·5 M-tris buffer, pH 8·0. After incubation at 37° for the times shown, 0·2 ml. samples were removed and incubated for 30 min. at 37° with 0·5 ml. of a mixture containing heat-denatured DNA (0·5 mg.), glycine buffer, pH 9·2 (0·1 M), $MgCl_2$ (0·03 M) and NaCl (5 mM). The final pH was 8·8. △: (*a*) 40 min. after infection with T2r (4 mg. of protein/ml. of extract); (*b*) 32 min. after infection with T5 (2 mg. of protein/ml.). ○: Extracts from comparable uninfected cultures. ▲, ●: As △ and ○ respectively but no ribonuclease added.

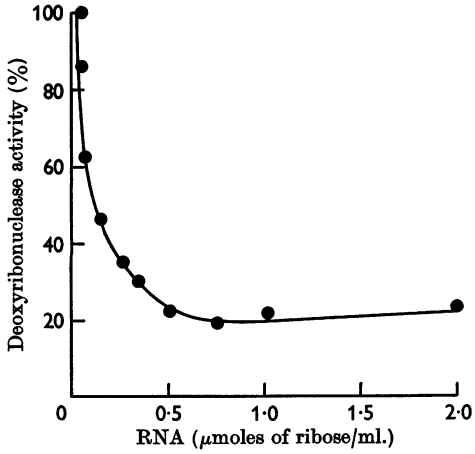

Fig. 5. Effect of *Escherichia coli* ribonucleic acid on the activity of *Escherichia coli* deoxyribonuclease. Deoxyribonuclease (0·3 ml., see the Materials and Methods section), phosphate buffer, pH 7·0 (8 mM), $MgSO_4$ (31 mM), DNA (0·6 mg.) and RNA (as indicated) were incubated in a volume of 1·3 ml. for 45 min.

Table 2. *Inhibition of* Escherichia coli *deoxyribonuclease by heated extracts of uninfected and bacteriophage-infected* Escherichia coli

Ultrasonic extracts prepared for the experiments of Figs. 1 and 2 were heated at 100° for 40 min. to denature endogenous nucleases. Precipitated protein was removed by centrifuging. The effect on *E. coli* deoxyribonuclease was tested as described in Fig. 5. Except for the controls, each tube contained 0·1 ml. of the boiled extract (0·07 μmole of ribose in Expt. 1; 0·13 μmole in Expt. 2).

Expt. no.	Phage	Time after infection (min.)	Inhibition of deoxyribonuclease (%)
1	None	—	50
	T2r	3	47
		6	47
		10	51
		15	46
2	None	—	58
	T6r+	6	52
		14	56
		24	57

thesis in relation to other processes which occur after infection with T5 was investigated, since this phage causes most of the bacterial DNA to be extensively degraded several minutes before the synthesis of bacteriophage DNA is apparent (Murray & Whitfield, 1953; Manson, 1957; Pfefferkorn & Amos, 1958; Crawford, 1959). In our experiments with either strain B or strain B/2 as host bacteria, the increase of deoxyribonuclease activity did not start until most of the bacterial DNA had been broken down. From the experiment in Fig. 7, it appears that the increase occurs slightly after the increase in the activity of the enzyme system which phosphorylates thymidylate.

DISCUSSION

Our results disagree with those reported by Kozloff (1953), who concluded that the increased deoxyribonuclease activity in extracts of T6-infected *E. coli* is due to the destruction of an inhibitory component of the bacterial RNA. We are not able to explain the differences in the results. In our experiments, treatment with pancreatic ribonuclease increased the deoxyribonuclease ac-

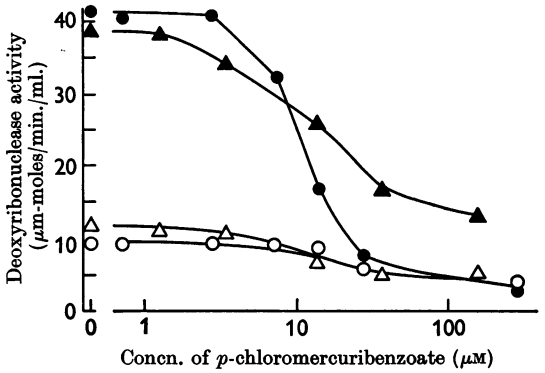

Fig. 6. Effect of *p*-chloromercuribenzoate on deoxyribonuclease activities. Extract (16 ml.), tris buffer, pH 8·0 (23 mM) and pancreatic ribonuclease (1 mg.) were incubated in a total volume of 17·4 ml. for 30 min. For the assay of deoxyribonuclease, ribonuclease-treated extract (0·3 ml.), glycine buffer, pH 9·2 (63 mM), $MgCl_2$ (19 mM), heat-denatured DNA (1 mg.) and sodium *p*-chloromercuribenzoate (as indicated) were incubated in a volume of 1·6 ml. for 60 min. The final pH was 8·8. ●, Extract from *E. coli* B 15 min. after infection with T2r$^+$; ▲, 32 min. after infection with T5; ○, △, uninfected portions of cultures ● and ▲ respectively.

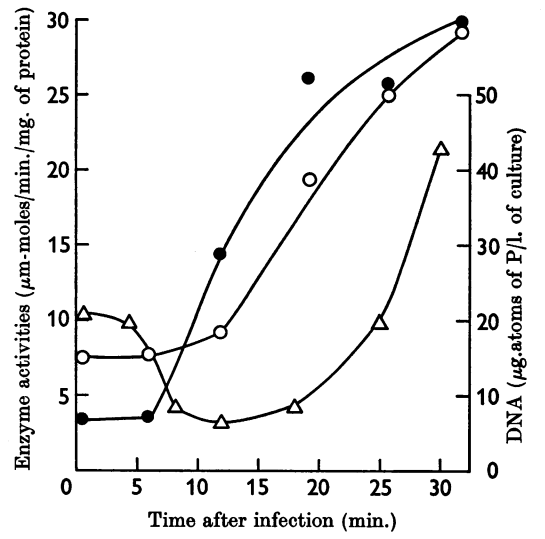

Fig. 7. Comparison of net changes of deoxyribonucleic acid with changes of deoxyribonuclease and thymidylate-kinase activities of *E. coli* B/2 infected with T5. △, DNA; ●, thymidylate kinase; ○, deoxyribonuclease (activity measured as described in Fig. 4, after incubation for 20 min. with ribonuclease). To ensure that enzyme synthesis was stopped promptly at the time of sampling, chloramphenicol (25 μg./ml.) was added to the mixtures of culture and ice and also on resuspending the bacteria in 0·01 M-phosphate buffer, pH 7·3.

tivities in extracts from both infected and uninfected bacteria. The resulting activities were still very much greater in the infected extracts than in those obtained before infection. The inhibitory activities of extracts in which the endogenous nucleases had been denatured confirmed the presence of the inhibitory RNA but gave no evidence for its destruction after infection. We conclude that the increase of deoxyribonuclease activity is due to the synthesis of one or more enzymes in the infected bacteria. This is consistent with the fact that chloramphenicol and thienylalanine prevent the rise in deoxyribonuclease activity (Kunkee & Pardee, 1956). There is no reason to believe that chloramphenicol acts by preventing the destruction of inhibitory RNA since we have shown that it also prevents the rise in deoxyribonuclease activity if this is measured after the extracts have been treated with ribonuclease.

In all instances examined (see Levin & Burton, 1961), phages T2, T4 and T6 prevent the synthesis of normal bacterial proteins after infection. The concept of 'messenger RNA' can explain this as a result of the breakdown of the bacterial nucleus (Luria & Human, 1950; Luria, 1962) which also occurs after infection by phage T5 (Murray & Whitfield, 1953). These arguments indicate that all the proteins made by bacteria infected with these phages are structurally determined by the phage and not by the bacteria. Apart from the proteins of the phage particles themselves, several enzyme proteins which are synthesized after infection are not detected in the uninfected bacteria (Bessman & Bello, 1961; Aposhian & Kornberg, 1962; Greenberg, Somerville & De Woolf, 1962; and Hogness, 1962, for earlier references).

The effects of *p*-chloromercuribenzoate indicate differences between the deoxyribonucleases produced after infection by phages T2 and T5. This is consistent with the view that they are structurally determined by the phages, although in such respects as the requirement for a bivalent metal ion, optimum pH and a preference for heat-denatured DNA they resemble the alkaline phosphodiesterase of normal *E. coli* (Lehman, 1960).

There is general agreement that after infection by phages T2 and T6 the increase of deoxyribonuclease activity occurs at about the same time as those reported for the synthesis of the enzymes concerned in DNA synthesis. In our experiments this is also true for T5 and the increase of deoxyribonuclease is first detectable at a time when most of the bacterial DNA has been degraded. Although Crawford (1959) reported that the increase of deoxyribonuclease activity occurred sooner after the infection with T5, he discovered that the addition of citrate at the time of infection did not prevent the breakdown of the bacterial DNA

and yet it prevented the increased deoxyribonuclease activity. This observation agrees with our evidence that the deoxyribonuclease activity produced by infection with T5 is not required for the breakdown of the bacterial DNA.

The finding that the enzyme appears after the breakdown of the bacterial DNA supports the above argument that it is not a normal bacterial protein and that its structure is determined by the phage DNA.

As is discussed by Kunkee & Pardee (1956), there is evidence to suggest that the enzyme may be concerned in the degradation of the DNA of the superinfecting phages, but the effects of streptomycin suggest that the process may not be essential for phage multiplication. There may therefore be another function of the enzyme but it is as yet obscure.

SUMMARY

1. Evidence is presented that the high deoxyribonuclease activity in extracts made from *Escherichia coli* infected with phages T2, T5 or T6 is due to the synthesis of one or more enzymes after infection. Previous claims that the increased activities were due to phage-induced degradation of an RNA component which inhibits deoxyribonuclease were not confirmed.

2. The increase due to phage-infection was greatest when deoxyribonuclease activity was measured at about pH 9 with heat-denatured DNA as substrate. Under these conditions, increases in activity of up to ninefold were observed as a result of infection by phage T5.

3. The deoxyribonuclease activity after infection with T2 was markedly more sensitive to inhibition by 30 μM-*p*-chloromercuribenzoate than that before infection, or that after infection with T5.

4. In T5-infected cultures, the increase of deoxyribonuclease activity did not start until most of the bacterial DNA had been degraded to acid-soluble materials. The increase occurred at about the same time as the increase of the activity responsible for the phosphorylation of thymidylate.

A. B. S. gratefully acknowledges a scholarship from the Medical Research Council.

REFERENCES

Adams, M. H. (1950). *Meth. med. Res.* **2**, 1.
Aposhian, H. V. & Kornberg, A. (1962). *J. biol. Chem.* **237**, 519.
Bessman, M. J. & Bello, L. J. (1961). *J. biol. Chem.* **236**, PC72.
Burton, K. (1955). *Biochem. J.* **61**, 473.
Burton, K. (1956). *Biochem. J.* **62**, 315.
Crawford, L. V. (1959). *Virology*, **7**, 359.
Daly, M. M., Allfrey, V. G. & Mirsky, A. E. (1949). *J. gen. Physiol.* **33**, 497.
Dische, Z. (1930). *Mikrochemie*, **8**, 4.
Emanuel, C. F. & Chaikoff, I. L. (1953). *J. biol. Chem.* **203**, 167.
Fessler, L. I., Kelemen, M. V. & Burton, K. (1960). *Biochem. J.* **77**, 558.
Greenberg, G. R., Somerville, R. L. & De Woolf, S. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 242.
Hogness, D. S. (1962). In *The Molecular Control of Cellular Activity*, 1st ed., p. 189. Ed. by Allen, J. M. New York: McGraw-Hill Book Co. Inc.
Hughes, D. E. (1951). *Brit. J. exp. Path.* **32**, 97.
Hughes, D. E. (1961). *J. biochem. microbiol. Technol. Engng*, **3**, 405.
Kirby, K. S. (1956). *Biochem. J.* **64**, 405.
Kozloff, L. M. (1953). *Cold. Spr. Harb. Symp. quant. Biol.* **18**, 209.
Kunkee, R. E. & Pardee, A. B. (1956). *Biochim. biophys. Acta*, **19**, 236.
Lanni, Y. T. (1958). *Virology*, **5**, 481.
Lehman, I. R. (1960). *J. biol. Chem.* **235**, 1479.
Lehman, I. R., Roussos, G. & Pratt, E. A. (1962). *J. biol. Chem.* **237**, 829.
Levin, A. P. & Burton, K. (1961). *J. gen. Microbiol.* **25**, 307.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
Luria, S. E. (1962). *Science*, **136**, 685.
Luria, S. E. & Human, M. L. (1950). *J. Bact.* **59**, 551.
Manson, L. A. (1957). *Bact. Proc.* p. 37.
Mejbaum, W. (1939). *Hoppe-Seyl. Z.* **258**, 117.
Murray, R. G. E. & Whitfield, J. F. (1953). *J. Bact.* **65**, 715.
Ogur, M. & Rosen, G. (1950). *Arch. Biochem.* **25**, 262.
Pardee, A. B. & Williams, I. (1952). *Arch. Biochem. Biophys.* **40**, 222.
Pardee, A. B. & Williams, I. (1953). *Ann. Inst. Pasteur*, **84**, 147.
Pfefferkorn, E. & Amos, H. (1958). *Virology*, **6**, 299.
Putnam, F. W., Kozloff, L. M. & Niel, J. C. (1949). *J. biol. Chem.* **179**, 303.
Stone, A. B. & Burton, K. (1961). *Biochem. J.* **81**, 2P.