acid in the gland than to the metabolic and synthetic activities of the tissue.

We wish to express our thanks to the Medical Research Council for the grant of a studentship to one of us (D. Y. W.) during the tenure of which this work was done.

REFERENCES

- Aminoff, D., Morgan, W. T. J. & Watkins, M. W. (1952). *Biochem. J.* **51**, 379.
- Brody, S. & Nisbet, R. (1938). *Res. Bull. Mo. agric. Exp. Sta.* no. 285.
- Cox, W. M., jun. & Mueller, A. J. (1937). *J. Nutr.* **13**, 249.
- Denamur, R., Fauconneau, G. & Guntz, G. (1958). *C.R. Acad. Sci., Paris*, **246**, 652.
- Dische, Z. (1947). *J. biol. Chem.* **167**, 189.
- Folley, S. J. & French, T. H. (1950). *Biochem. J.* **46**, 465.
- Forrest, R. J., Wilken, D. R. & Hansen, R. G. (1960). *Biochim. biophys. Acta*, **37**, 551.
- Greenbaum, A. L. & Slater, T. F. (1957a). *Biochem. J.* **66**, 148.
- Greenbaum, A. L. & Slater, T. F. (1957b). *Biochem. J.* **66**, 155.
- Hurlbert, R. B., Schmitz, H., Brumm, A. F. & Potter, V. R. (1954). *J. biol. Chem.* **209**, 23.
- Kirkham, W. R. & Turner, C. W. (1953). *Proc. Soc. exp. Biol., N.Y.*, **83**, 123.
- McLean, P. (1958a). *Biochim. biophys. Acta*, **30**, 303.
- McLean, P. (1958b). *Biochim. biophys. Acta*, **30**, 316.
- Manson, W. (1956). *Biochim. biophys. Acta*, **19**, 398.
- Park, J. T. & Johnson, M. J. (1949). *J. biol. Chem.* **181**, 149.
- Reid, E. & Stevens, B. M. (1957). *Biochem. J.* **67**, 262.
- Shimizu, H. (1957). *Tohoku J. agric. Res.* **7**, 339.
- Slater, T. F. & Planterose, D. N. (1958). *Biochem. J.* **69**, 417.

Biochem. J. (1960) **77**, 558

Synthesis of Protein in a Purine-Requiring *Escherichia coli* Infected with Bacteriophage T2

By LISELOTTE I. FESSLER, MARIANNE V. KELEMEN* AND K. BURTON
Medical Research Council Cell Metabolism Research Unit, Department of Biochemistry,
University of Oxford

(Received 4 April 1960)

Strain B-96 is a mutant of *Escherichia coli* which requires adenine, guanine, hypoxanthine or xanthine for growth (Gots, 1950, 1953). Infection with T2r bacteriophage does not appear to alter the defect, since in the absence of adenine the deoxyribonucleic acid content of the culture remains constant and few bacteriophages are formed (Burton, 1955). This paper presents evidence that *E. coli* B-96 infected with T2r bacteriophage can, in the absence of exogenous adenine, synthesize bacteriophage-specific proteins and the enzymes required for the synthesis of bacteriophage deoxyribonucleic acid. The addition of adenine is, however, required for net synthesis of deoxyribonucleic acid.

METHODS AND MATERIALS

Bacteria. *E. coli* B-96 was obtained from Dr J. S. Gots. The bacteria were grown at 37° with aeration in the following growth medium (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 , 10 g. of mannitol, 30 mg. of adenine and 15 mg. of L-histidine hydrochloride. These concentrations of adenine and histidine are in the optimum range for growth.

* Present address: Department of Chemistry, King's College, Newcastle upon Tyne 1.

Bacteriophage. The T2r strain was prepared and assayed as described by Adams (1950) and Burton (1955).

isoCitric dehydrogenase. A partially purified preparation was kindly given by Dr H. L. Kornberg.

Nucleotides. Deoxyribonucleotides were obtained from the California Corporation for Biochemical Research (Los Angeles 63, Calif., U.S.A.) and other nucleotides from Sigma Chemical Co. (St Louis 18, Mo., U.S.A.).

Conditions of bacterial growth and bacteriophage infection. *E. coli* B-96 was grown on agar slants overnight at 37°. The growth medium, described above, was inoculated from this culture, aerated overnight at 37° and then added to 7 vol. of fresh growth medium. Aeration was continued until the concentration of cells was about 5×10^8 /ml. (2–2½ hr.). The bacteria were centrifuged, washed at 4° with the original volume of buffered saline (Burton, 1955) and resuspended in growth medium at 37° in the absence of adenine. The bacteria were infected with an average of five infective T2r particles per cell and aeration was continued. Thereafter adenine was added as indicated.

The medium used to suspend the bacteria in the experiment of Table 3 had a low concentration of phosphate and contained (per l.) 10 g. of mannitol, 3 g. of NaCl , 27 mg. of KH_2PO_4 , 10 mg. of MgSO_4 , 0.16 mg. of FeCl_3 and 15 mg. of histidine hydrochloride and was buffered with 0.05 M-triethanolamine (adjusted to pH 7.4 with HCl) and 0.01 M-succinic acid (adjusted to pH 7.4 with NH_3).

Some difficulty was caused by unexplained variations in the growth rate of the bacteria. For the experiments which are described in detail, the cultures reached the required

concentration in 2–2½ hr. after inoculation from the overnight culture. Certain cultures for unknown reasons required more than 4 hr. Although they appeared to give normal growth of phage in the presence of adenine, they synthesized protein very slowly when infected with phage in the absence of adenine.

Preparation of cell-free extracts. Portions of uninfected and infected cultures were chilled and the bacteria were sedimented rapidly at 4°. The bacteria were suspended in 0.1M-potassium phosphate buffer, pH 7.0, (2 ml./100 ml. of original culture) and disrupted for 3 min. in a Mullard-MSE ultrasonic disintegrator working at 90 kcyc./sec. and 60 w with a 1.9 cm. diam. stainless-steel parallel probe. The suspension was centrifuged at 3000 g for 15 min. The supernatant, designated 'cell-free extract', was stored at -20° until required.

Enzyme assays

Deoxycytidylate hydroxymethylase. This was assayed according to Flaks & Cohen (1959) with the inclusion of di- and tri-phosphopyridine nucleotide (each at 5 mM), 2.5 mM-DL-isocitrate and isocitrate dehydrogenase (0.1 mg./ml.). These additions slightly increased the activity, perhaps because they kept tetrahydropteroylglutamic acid in the reduced form. The reaction was stopped by addition of perchloric acid to a final concentration of 0.2N; the acid-soluble fraction was neutralized with KOH. Potassium perchlorate was removed by centrifuging and portions of the supernatant were hydrolysed and plated as described by Flaks & Cohen (1959). The radioactivity was measured in a gas-flow proportional counter fitted with a thin Micromil window (Nuclear-Chicago Corp., Chicago 10, Ill., U.S.A.).

Deoxynucleotide kinases. For the assay of hydroxymethylcytidylate kinase (and also the deoxyribonucleotide polymerase), [¹⁴C]hydroxymethylcytidylate was prepared enzymically according to Flaks & Cohen (1959) and purified by chromatography on Dowex-1 formate resin. The specific activity was calculated from the measured radioactivity and ultraviolet absorption, from the value of 13.5×10^3 at 284 mμ and pH 1 for the molar extinction coefficient. The assay mixture contained 0.02 μmole of [¹⁴C]hydroxymethylcytidylate (500 000 counts/min./μmole), 1.0 μmole of adenosine triphosphate (ATP), 4 μmoles of 2-amino-2-hydroxymethylpropane-1:3-diol brought to pH 7.8 with HCl, 1.0 μmole of MgCl₂ and enzyme containing 5–15 μg. of protein in a total volume of 0.1 ml. After 10 min. at 37°, the reaction was stopped by adding perchloric acid to a final concentration of 0.4N. The acid soluble fraction was neutralized with KOH and the resulting potassium perchlorate was removed. The labelled nucleotides were separated on 2 cm. × 0.8 cm. diam. columns of Dowex-1 × 8 formate resin. First the monophosphate was eluted by 0.1N-formic acid, then the diphosphate by 4M-formic acid and finally the triphosphate by 2N-HCl. The radioactivity in each fraction was determined. The ratio of the di- to triphosphate was found to vary with experimental conditions, but was always less than 0.5.

The other deoxyribonucleotide kinases were assayed by incubating 2.5 μmoles of thymidylate, deoxyguanylate or deoxyadenylate with a mixture of 100 μmoles of 2-amino-2-hydroxymethylpropane-1:3-diol adjusted to pH 7.8 with HCl, 10 μmoles of ATP, 10 μmoles of MgCl₂ and enzyme containing 0.3–1.0 mg. of protein in a total volume of 2.5 ml. Controls without deoxyribonucleotides were included. After 10 min. at 37° the reaction was stopped as

described above. For the assay of the deoxyguanylate or thymidylate kinases the acid-soluble fraction was chromatographed on a 5.5 cm. × 0.8 cm. diam. column of Dowex-1 × 8 formate resin. All nucleotides, except deoxyguanosine triphosphate or thymidine triphosphate, were eluted with 4M-formic acid plus 0.3M-ammonium formate (about 70–80 ml.). The deoxyguanosine triphosphate or thymidine triphosphate was then eluted with M-ammonium formate (pH 5) and estimated spectrophotometrically. The deoxyribonucleoside diphosphates were usually not investigated because of the more tedious chromatography that is required for their separation. In one experiment they were isolated and found to represent about 10% of the triphosphates. For the assay of the deoxyadenylate kinase the deoxyadenosine triphosphate was determined after the ribonucleotides had been removed by periodate oxidation according to Lehman, Bessman, Simms & Kornberg (1958). The acid-soluble fraction was chromatographed on Dowex-1 × 8 formate columns, 5.5 cm. × 0.8 cm. diam. The ultraviolet-absorbing components except deoxyadenosine triphosphate were eluted by about 50 ml. of 4M-formic acid plus 0.1M-ammonium formate. Deoxyadenosine triphosphate was then eluted by 4M-formic acid plus 0.3M-ammonium formate.

Deoxyribonucleotide polymerase. This enzyme was assayed according to Lehman *et al.* (1958) by measuring the incorporation of [¹⁴C]hydroxymethylcytidylate (500 000 counts/min./μmole) into deoxyribonucleic acid (DNA) in the presence of deoxyadenylate, deoxyguanylate, thymidylate and calf-thymus DNA which had been heated for 5 min. at 100° and pH 9. The incubation mixture contained 0.02 μmole of each deoxynucleotide, 3 μmoles each of ATP and MgCl₂, 15 μmoles of 2-amino-2-hydroxymethylpropane-1:3-diol adjusted to pH 7.8 with HCl, 0.1 mg. of DNA and enzyme containing 0.05–0.1 mg. of protein in a total volume of 0.3 ml. The incubation was for 30 min. at 37°. Under these conditions the deoxyribonucleoside monophosphates were converted into the triphosphates by the respective kinases and subsequently polymerized. The rates of phosphorylation are much higher than the rates of polymerization.

In all enzyme assays two levels of enzyme were used and the reactions were proportional to the enzyme concentration.

Isolation of ³⁵S-labelled protein

Portions (10 ml.) of the culture were precipitated with perchloric acid, washed with 5% trichloroacetic acid, extracted twice at 70° for 20 min. with 0.5N-perchloric acid and washed twice with 0.5N-perchloric acid. The residue was suspended in acetone, plated on filter paper and counted with an end-window Geiger-Müller tube.

Radioactive carbon in nucleic acid purines. Portions of the cultures (10 ml.) were added to 1 ml. of 55% (w/v) trichloroacetic acid. The precipitate was washed several times with 5% (w/v) trichloroacetic acid. Nucleic acids were extracted from it by 0.5N-perchloric acid at 70°, the purines were obtained as their silver salts (cf. McDonald, 1954) and plated on filter paper for radioactivity measurement by an end-window Geiger-Müller tube. After counting, the silver salts were decomposed by 0.1N-HCl and the amounts of purine determined spectrophotometrically (Loring, 1955). Paper chromatography in propan-2-ol-HCl (Wyatt, 1951) confirmed that adenine and guanine were the only ultraviolet-absorbing materials present. Under the

conditions used the counting rates of different samples from the same culture were approximately proportional to the amounts of purines.

Chemical analyses

DNA was estimated on 5 ml. portions of the cultures as described by Burton (1956) with deoxyribose as the standard. The apparent (i.e. purine-bound) deoxyribose was multiplied by 2 to give values for the total deoxyribose. Protein was determined according to Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Synthesis of purines

To test for the completeness of the genetic block in strain B-96, [^{14}C]glycine (5.4 mg. = 0.02 mc/l.) was added to normal and phage-infected bacteria in adenine-free medium. After 60 min., portions (10 ml.) of the cultures were taken and the radioactivities of the silver salts of the nucleic acid purines were measured. After correction for background radiation, the specific activities and standard deviations were 12.3 ± 7.6 counts/min./ μmole in the uninfected culture and 16.9 ± 11.8 counts/min./ μmole in the phage-infected culture. Analogous experiments with *E. coli* strain B indicated that activities of at least 1500 counts/min./ μmole would have been found with this strain. There is therefore negligible incorporation of glycine into purines by strain B-96, although it is incorporated into protein at rates which are comparable with those in the parent strain B.

Fig. 1. Dependence of net DNA synthesis in infected cultures on the presence of adenine. At 0 min. two 50 ml. portions of washed cells were infected with phage T2. Adenine (30 mg./l.) was added to one portion. The cultures were aerated at 37°. ○, Adenine; ●, no added adenine.

Synthesis of deoxyribonucleic acid and proteins

As shown in Fig. 1, omission of adenine from the infected culture prevents an increase in the DNA content. This observation led us to investigate whether exogenous adenine is required solely for the net synthesis of DNA or whether it is also needed for the synthesis of ribonucleic acid (RNA) and protein. Fig. 2 shows that the absence of adenine decreases the incorporation of ^{35}S into the total proteins of the infected culture by only about 20%. A similar result has been reported by Watanabe & Kiho (1958) in an experiment with another purine-requiring mutant of *E. coli*.

In the experiment in Fig. 3 the infected cultures were incubated in the absence of adenine for periods of 8 or 15 min. and then adenine was added together with chloramphenicol, which is expected to arrest any further protein synthesis. Under these conditions net synthesis of DNA occurred, indicating that the proteins needed for the synthesis of phage DNA were formed in the absence of adenine, although, as suggested by the experiment in Fig. 3(a), the synthesis of the proteins may have been somewhat slower than in the presence of adenine.

Further evidence that these proteins are made in the absence of exogenous adenine is given by the

Fig. 2. Incorporation of ^{35}S into the total proteins of infected cells in the presence and absence of adenine. A washed culture was infected with an average of eight T2r particles/cell in a growth medium free of adenine and containing 10 mg. of MgCl_2 and 115 mg. of Na_2SO_4 /l. instead of MgSO_4 . Carrier-free $\text{Na}_2^{35}\text{SO}_4$ (0.18 mc/l.) was added 3 min. later and the culture was divided. One sample was incubated in the absence of adenine (○). After 40 min. a portion of the sample was removed, adenine (30 mg./l.) was added and the incubation was continued (▲). To another sample adenine (30 mg./l.) was added immediately after the $\text{Na}_2^{35}\text{SO}_4$ (●).

Table 1. *Base composition of deoxyribonucleic acid of Escherichia coli B-96 infected with T2r phage in the absence of adenine*

Analyses were made according to Hershey, Dixon & Chase (1953) on the DNA from portions (200 μ l.) of culture both before and at various times after infection with phage in the absence of adenine. No explanation can be given for the low recoveries of adenine. Separate determinations on the same culture showed that the amount of DNA remained constant. The amounts of the bases are expressed as μ moles recovered/100 μ moles of total deoxyribose.

Time after infection (min.)	Hydroxymethylcytosine	Cytosine	Guanine	Thymine	Adenine	Base recovery (%)
0	0	24.7	23.6	26.0	17.7	92
15	1.3	22.7	25.0	26.7	18.3	94
30	3.7	15.7	22.3	27.7	18.8	88
45	6.7	11.0	21.0	29.7	20.0	88

Fig. 3. Synthesis of DNA when adenine and chloramphenicol are added at different times after infection. In the same experiment as shown in Fig. 1, additional portions of the infected culture were aerated at 37°. Adenine (30 mg./l.) was added as indicated. Chloramphenicol (20 mg./l.) was added at 8 and 15 min. after infection in (a) and (b) respectively. To other portions of the culture not shown in the figure, chloramphenicol was added at 3 min. after infection and adenine at either 0 or 3 min. No change occurred in the DNA content.

conversion of bacterial DNA into phage DNA. This is indicated by the results in Table 1, which show a loss of cytosine and the appearance of hydroxymethylcytosine in the DNA of infected cells.

More direct evidence that some of the necessary enzymes are formed in the absence of exogenous adenine is presented in Fig. 4 and Table 2. Whether adenine is present or not the enzyme levels reach a similar maximal level, although the rate of enzyme synthesis is somewhat slower in the absence of adenine.

For undefined reasons some cultures of *E. coli* B-96 grew more slowly than normal cultures. If they were infected in the presence of adenine when they had reached a concentration of about 5×10^8 cells/ml. they synthesized DNA and the necessary enzymes at about the same rate as described above.

Fig. 4. Level of deoxyguanylate kinase in *Escherichia coli* B-96 infected with T2r bacteriophage in the presence (●) and absence (○) of adenine. Assay was as described in the Methods section.

However, in the absence of adenine the enzymes began to be made only after a much longer period of infection. Similarly, if cultures grown at the normal rate were kept in an adenine-free medium for 5 min. before infection the synthesis of protein was markedly reduced in the cultures devoid of exogenous adenine.

Synthesis of a structural protein of the phage was examined by measuring the serum-blocking power essentially according to DeMars (1955). Whether adenine was present or not, the levels detected were of the same order of magnitude and appreciable serum-blocking power was first observed between 20 and 30 min. after infection. The results are not presented in detail because they were variable and do not permit strictly quantitative comparisons.

Ribonucleic acid synthesis

A number of the 'early enzymes' are not found in the host bacteria, and, like the structural proteins of the phage, they are probably new proteins synthesized after the infection (Flaks & Cohen, 1957, 1959; Kornberg, Zimmerman, Kornberg & Josse, 1959; Koerner, Smith & Buchanan, 1959; Somerville, Ebisuzaki & Greenberg, 1959; Bessman, 1959; Bessman & Van Bibber, 1959). If RNA plays a role in the synthesis of proteins (review Loftfield, 1958) it is expected that new RNA would be synthesized in the infected cultures grown in the absence of adenine from precursors contained in the bacteria. Volkin & Astrachan (1956) and Astrachan & Volkin (1959) demonstrated that, although phage infection arrests net RNA synthesis, there is RNA turnover which appears to involve the synthesis of a different kind of RNA, as shown by an altered distribution of ^{32}P among the four nucleotides. This

effect of the phage is most marked in the presence of chloramphenicol. As is shown in Table 3, it occurs in cultures which are devoid of exogenous adenine, although these cultures incorporated less ^{32}P into RNA (about one-seventh of that incorporated in the presence of adenine). Other experiments in the absence of chloramphenicol indicated that infection in the absence of adenine caused a similar change in the distribution of ^{32}P among the nucleotides obtained from the RNA, and there was also a reduction to about one-third of the incorporation in the presence of adenine.

Watanabe & Kiho (1958) have also reported that the changed pattern of incorporation occurs in the absence of adenine. It suggests that exogenous adenine is not required for the synthesis of a new type of RNA molecule. Although the lower incorporation of ^{32}P in the absence of adenine might suggest a slower turnover of RNA, it could also be due to a decreased entry of ^{32}P into the nucleotide precursors of RNA.

Table 2. *Level of enzymes involved in the synthesis of phage deoxyribonucleic acid*

The activities of all the enzymes shown except deoxyadenylate kinase were measured on the same five cell-free extracts by methods described in the text.

Enzyme	Basis of assay	Enzyme activities ($\mu\text{moles/mg. of protein/hr.}$)				
		Uninfected	8 min. after infection		15 min. after infection	
			No adenine	Adenine	No adenine	Adenine
Deoxycytidylate hydroxymethylase	Incorporation of $\text{H} \cdot ^{14}\text{CHO}$	0	0.20	0.20	0.30	0.30
Deoxyribonucleotide kinases	Formation of					
Hydroxymethylcytidylate	Di- and tri-phosphate	0	0.78	1.50	2.58	2.58
Thymidylate	Triphosphate	0.47	0.91	1.89	3.39	3.05
Deoxyguanylate	Triphosphate	0.65	1.28	2.42	4.09	3.66
Deoxyadenylate	Triphosphate	3*	—	—	—	—
Deoxyribonucleotide polymerase	Incorporation of $[^{14}\text{C}]$ -hydroxymethylcytidylate	Nil†	0.001	0.004	0.007	0.006

* The activity was also approximately the same at 5 and 12 min. after infection.

† $[^{14}\text{C}]$ Hydroxymethylcytidylate is not phosphorylated and is therefore not incorporated into DNA.

Table 3. *Incorporation of ^{32}P -labelled orthophosphate into ribonucleic acid*

Chloramphenicol (4.5 mg.) was added to a 150 ml. portion of a washed culture in low-phosphate medium devoid of adenine. After 3 min. a tracer dose of carrier-free $\text{Na}_2\text{H}^{32}\text{PO}_4$ (0.066 mc) was added and 10 min. later the culture was chilled and centrifuged. Another 150 ml. portion of the washed culture was infected with T2r phage. After 3 min., 4.5 mg. of chloramphenicol was added; 6 min. after infection 0.066 mc of $\text{Na}_2\text{H}^{32}\text{PO}_4$ was added and 10 min. later the cells were chilled and centrifuged. The RNA nucleotides were isolated as described by Hecht & Potter (1956).

Nucleotide	Specific activities			
	Counts/min./ μmole		Relative to guanylic acid	
	Uninfected	Infected	Uninfected	Infected
Guanylic acid	2820	3310	(1.0)	(1.0)
Cytidylic acid	3620	3910	1.3	1.2
Adenylic acid	3590	5980	1.3	1.8
Uridylic acid	3750	6570	1.3	2.0

DISCUSSION

The results indicate that, after phage infection, the enzymes required for the synthesis of phage DNA (Flaks & Cohen, 1957, 1959; Koerner *et al.* 1959; Kornberg *et al.* 1959; Somerville *et al.* 1959; Bessman, 1959) are made whether exogenous purines are supplied or not. The extent of phage DNA synthesis is, however, controlled by the supply of preformed purines. In the absence of an exogenous purine source limited phage DNA synthesis can occur. Under these conditions some of the components of the bacterial DNA, which is degraded after infection, are apparently utilized for the synthesis of the new type of DNA, but a further supply of purines derived from other bacterial components does not seem to be available for DNA synthesis, since there is no net change in the DNA concentration.

The results obtained are not inconsistent with current concepts of the role of RNA in protein synthesis (review Loftfield, 1958) since the new RNA appears to be formed even under conditions where the purine supply is limited and where there is no net synthesis of nucleic acids.

SUMMARY

1. When a purine-requiring mutant (B-96) of *Escherichia coli* is infected with T2r phage, net synthesis of deoxyribonucleic acid requires a supply of exogenous purine. Direct and indirect evidence is presented that exogenous purines are not required for the synthesis of either the structural proteins of the phage or the enzymes necessary for the synthesis of phage deoxyribonucleic acid.

2. Even when exogenous adenine is not supplied, the nucleic acids of the bacterial cell undergo conversions which characteristically follow infection with phage T2. Cytosine of the deoxyribonucleic acid is replaced by 5-hydroxymethylcytosine and ³²P-labelled inorganic phosphate is incorporated into ribonucleic acid with an altered distribution among the nucleotides obtained by alkaline hydrolysis.

We wish to thank Professor Sir Hans Krebs, F.R.S., for his interest and for offering hospitality in his laboratories to L.I.F., and to Mrs E. M. Smith for technical assistance. L.I.F. held a Postdoctoral Fellowship from the National Science Foundation, U.S.A., and M.V.K. was a training scholar of the Medical Research Council.

REFERENCES

- Adams, M. H. (1950). *Meth. med. Res.* 2, 1.
 Astrachan, L. & Volkin, E. (1959). *Biochim. biophys. Acta*, 32, 449.
 Bessman, M. J. (1959). *J. biol. Chem.* 234, 2735.
 Bessman, M. J. & Van Bibber, M. J. (1959). *Biochem. Biophys. Res. Commun.* 1, 101.
 Burton, K. (1955). *Biochem. J.* 61, 473.
 Burton, K. (1956). *Biochem. J.* 62, 315.
 DeMars, R. I. (1955). *Virology*, 1, 83.
 Flaks, J. G. & Cohen, S. S. (1957). *Biochim. biophys. Acta*, 25, 667.
 Flaks, J. G. & Cohen, S. S. (1959). *J. biol. Chem.* 234, 1501.
 Gots, J. S. (1950). *Arch. Biochem.* 29, 222.
 Gots, J. S. (1953). *Nature, Lond.*, 172, 256.
 Hecht, L. I. & Potter, V. R. (1956). *Cancer Res.* 16, 999.
 Hershey, A. D., Dixon, J. & Chase, M. (1953). *J. gen. Physiol.* 36, 777.
 Koerner, J. F., Smith, M. S. & Buchanan, J. M. (1959). *J. Amer. chem. Soc.* 81, 2594.
 Kornberg, A., Zimmerman, S. B., Kornberg, S. R. & Josse, J. (1959). *Proc. nat. Acad. Sci., Wash.*, 45, 772.
 Lehman, I. R., Bessman, M. J., Simms, E. S. & Kornberg, A. (1958). *J. biol. Chem.* 233, 163.
 Loftfield, R. B. (1958). *Progress in Biophysics and Biochemistry*. London: Pergamon Press Ltd.
 Loring, H. S. (1955). In *The Nucleic Acids*, vol. 1. Ed. by Chargaff, E. & Davidson, J. N. New York: Academic Press Inc.
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* 193, 265.
 McDonald, I. W. (1954). *Biochem. J.* 57, 566.
 Somerville, R., Ebisuzaki, K. & Greenberg, G. R. (1959). *Proc. nat. Acad. Sci., Wash.*, 45, 1240.
 Volkin, E. & Astrachan, L. (1956). *Virology*, 2, 149.
 Watanabe, I. & Kiho, Y. (1958). In *Proceedings of the International Symposium on Enzyme Chemistry, Tokyo and Kyoto*. London: Pergamon Press Ltd.
 Wyatt, G. R. (1951). *Biochem. J.* 48, 581.