JOURNAL OF BACTERIOLOGY
Vol. 87, No. 5, pp. 1011–1018 May, 1964

COMPARATIVE EFFECTS OF 5-FLUOROURACIL ON STRAINS OF *BACILLUS MEGATERIUM*

J. T. WACHSMAN[1], S. KEMP, AND L. HOGG

Department of Microbiology, University of Illinois, Urbana, Illinois

Received for publication 2 November 1963

ABSTRACT

WACHSMAN, J. T. (University of Illinois, Urbana), S. KEMP, AND L. HOGG. Comparative effects of 5-fluorouracil on strains of *Bacillus megaterium*. J. Bacteriol. **87**:1011–1018. 1964.—Growth of *Bacillus megaterium* strain KM is severely inhibited by 5-fluorouracil (FU). Both thymidine and uridine are required to overcome this inhibition. The addition of uridine alone to a FU-inhibited culture permits good ribonucleic acid (RNA) and protein synthesis for the first 2 hr, but rather poor deoxyribonucleic acid synthesis. Uridine enhances the bactericidal effect of FU, promoting a decrease in the viable count of from 4 to 5 decades in 5 hr. Death begins after a 1-hr lag and is accompanied by hydrolysis of RNA and cell lysis, commencing during the 2- to 5-hr interval. The combination of FU and uridine is not bactericidal, when a methionine auxotroph is deprived of its required amino acid. Substrains of KM, partially resistant to FU, were isolated. Strain T_2 requires only thymidine to overcome the inhibitory effects of FU, whereas strain FU/2 requires only uridine. With a uridine auxotroph of strain KM, FU partially replaces uridine by permitting a small, but reproducible, increase in the amount of protein.

Since the development of 5-fluorouracil (FU) as a tumor-inhibiting compound (Duschinsky, Pleven, and Heidelberger, 1957; Heidelberger et al., 1957), FU, its nucleoside (F-uridine), and its deoxyribonucleoside (FUDR), have been extensively studied. These analogues were found to be inhibitory for mammalian cells (Harbers, Chaudhuri, and Heidelberger, 1959), microorganisms (Scheiner, Kostelak, and Duschinsky, 1957), and plant cells (van Noort and Wallace, 1963). In most systems studied, both uracil and thymine are needed to overcome the inhibitory effects of high levels of FU.

FU and its derivatives were found to inhibit at least three different biosynthetic processes: deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and the bacterial cell wall. DNA synthesis is affected because the conversion of deoxyuridylate to thymidylate (thymidylate synthetase) is specifically inhibited by the 5′-monophosphate of FUDR (Flaks and Cohen, 1959). The critical site(s) involved in the inhibition of RNA synthesis is not as well defined. FU inhibits the incorporation of uracil into RNA (Harbers et al., 1959; Horowitz, Saukkonen, and Chargaff, 1960) and is itself incorporated into RNA, where it may replace up to 50% of the uracil residues (Gordon and Staehelin, 1959; Horowitz et al., 1960). Such RNA might be functionally defective. In addition, Sköld (1958) found that FU inhibits uridine synthesis and serves as a substrate for uridine phosphorylase. Finally, it was shown that FU inhibits cell-wall biosynthesis in *Escherichia coli* (Tomasz and Borek, 1960) and *Staphylococcus aureus* (Rogers and Perkins, 1960), where it causes the accumulation of cell-wall precursors, some of which contain FU instead of uracil.

The present work is a continuation of the study of the effect of analogues on the growth and metabolism of *Bacillus megaterium* (Mangalo and Wachsman, 1962). Data are presented on the comparative inhibitory effects of FU on strain KM and several substrains: a methionine auxotroph, a uridine auxotroph, and two mutants partially resistant to FU.

MATERIALS AND METHODS

B. megaterium strain KM was grown on a basal medium containing 1.0% glucose, 0.65% Na-L-glutamate, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.1% Na_2SO_4, 0.01% $MgSO_4 \cdot 7H_2O$, and 3×10^{-4} M ferric citrate (final pH, 7.0). For the growth of strains M_1 (methionine auxotroph) and 239 (uridine auxotroph), the basal medium was supplemented with L-methionine (1.0 μmole/ml) or uridine (0.5 μmole/ml).

Strains M_1 and 239 were derived from strain KM by enrichment with 8-azaguanine (Wachsman and Mangalo, 1962). Strain FU/2 was iso-

[1] Research Career Development Awardee of the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

lated by repeated subculture of strain KM on basal medium supplemented with FU, uridine, and limiting thymine. It grows well on the basal medium alone, or basal medium containing FU and uridine. Strain T_2 was isolated after repeated subculture of strain KM on the basal medium containing FU, thymidine, and limiting uridine. It grows on the basal medium alone, or when it is supplemented with FU and thymidine.

Cells were grown at 37 C in 250-ml Erlenmeyer flasks (10 to 40 ml of culture) containing attached colorimeter tubes. Vigorous aeration was provided with a rotary water-bath shaker. Turbidity was determined with a Klett-Summerson colorimeter (with filter no. 64).

For experiments on cell chemistries, samples were removed and precipitated with a final concentration of 5% trichloroacetic acid in an ice bath. After allowing 0.5 hr for flocculation to occur, the precipitate was removed by centrifugation at 0 C, and was washed twice with cold 5% trichloroacetic acid. The pellet was resuspended in 3.0 ml of 5% trichloroacetic acid and heated at 98 C for 30 min. After cooling in an ice bath for 30 min, the samples were centrifuged and the supernatant fractions were removed with a drawn-out pipette. The pellets were allowed to dissolve overnight at room temperature in 3 ml of 0.5 N NH_4OH, prior to assaying for protein, according to Lowry et al. (1951). The supernatant fractions were assayed for RNA by a modified orcinol reaction (Ashwell, 1957), with ribose as the standard; optical density (OD) was determined at 670 mμ-OD at 580 mμ. DNA was determined by the method of Burton (1956), with deoxyribose as a standard and determining $OD_{595}-OD_{650}$.

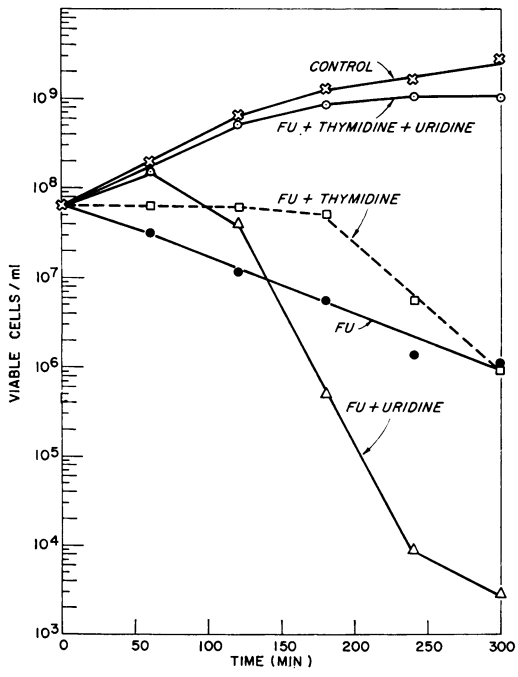

FIG. 1. *Effect of 5-fluorouracil (FU) on the viability of strain KM. FU and the above pyrimidines were added to portions of exponentially growing cells on basal medium, at a final concentration of 0.5 μmoles of each per ml. Incubation was at 37 C. Samples were removed and plated for viable count as described in Materials and Methods. Control = basal medium alone.*

For determinations of viable count, 1.0-ml samples were diluted 1 to 10 with basal medium, centrifuged, and the pellet resuspended with the aid of a vortex mixer. Samples (0.1 ml) of appropriate dilutions in the basal medium were spread on the surface of basal agar plates (basal medium containing 2% agar). Basal agar was routinely used, unless otherwise stated in the text. Colonies were counted after incubation for at least 48 hr at 35 C.

The basal medium was sterilized by autoclaving. All other compounds were sterilized by Millipore filtration, before addition to the basal medium.

All compounds used were commercial samples of analytical grade. We are indebted to W. E. Scott of Hoffmann-La Roche Inc., Nutley, N.J., for generous supplies of 5-fluorouracil, 5-fluorouridine, and 5-fluoro-2′-deoxyuridine. Thymidine and uridine were obtained from Mann Research Laboratories, Inc., New York, N.Y.

RESULTS

The effect of FU on the viability of strain KM is shown in Fig. 1. It is apparent that both thymidine and uridine are required to overcome the bactericidal effect of this analogue. However, the rate of increase in viable cells in the presence of all three pyrimidines is somewhat less than that observed on the basal medium alone (control). Thymidine is protective for approximately 3 hr, but, by 5 hr, the viable count in the presence of FU and thymidine is equal to the count with FU alone. The addition of uridine to a culture contain-

TABLE 1. *Effect of 5-fluorouracil (FU) on strain KM**

Additions to basal medium	Time (hr)	Relative increase			
		Turbidity	Protein	RNA	DNA
None	0	1.00	1.00	1.00	1.00
	0.5	1.60	1.77	1.80	1.77
	1	2.47	2.62	2.94	2.72
	2	5.18	6.53	6.50	6.16
	5	11.4	21.5	12.2	17.7
FU	0.5	1.40	1.33	1.20	1.23
	1	1.51	1.52	1.26	1.26
	2	1.62	1.71	1.19	1.21
	5	2.16	1.60	1.15	1.31
FU + uridine	0.5	1.61	1.40	1.82	1.33
	1	2.41	2.21	2.95	1.53
	2	4.59	5.15	5.50	1.98
	5	3.80	8.46	1.99	1.72
FU + thymidine	0.5	1.34	1.17	1.15	1.44
	1	1.41	1.37	1.13	1.74
	2	1.68	1.71	1.23	2.24
	5	2.22	1.51	1.41	2.54
FU + thymidine + uridine	0.5	1.54	1.51	1.86	1.76
	1	2.34	2.21	3.06	2.59
	2	4.91	5.95	6.93	6.05
	5	9.74	16.7	12.8	14.3

* Portions of a culture growing exponentially on the basal medium were supplemented with the above compounds at a final concentration of 0.5 μmoles of each per ml. Incubation was at 37 C. At zero time, the cultures contained the following amounts per ml: 93.6 μg of protein, 26.8 μg of RNA, 2.36 μg of DNA, and 6.6×10^7 viable cells.

ing FU results in an increase in the viable count for about the first hour, followed by a rapid rate of death. Uridine, therefore, greatly enhances the bactericidal action of FU and, in several experiments, induced a 4- to 5-decade kill in 5 hr.

Both RNA and DNA synthesis are severely inhibited by FU, and both uridine and thymidine are required to overcome this inhibition (Table 1). In the presence of all three pyrimidines, there is a small, but reproducible, increase (3 to 6%) in the rate of RNA synthesis over that observed on the basal medium alone, whereas the increases in DNA, protein, and turbidity occur at a lower rate. The addition of thymidine to a culture inhibited by FU causes a slight stimulation in RNA synthesis between 2 and 5 hr, approximately a 90% stimulation in DNA synthesis by 5 hr, and has little or no effect on protein synthesis. The addition of uridine to a culture inhibited by FU restores RNA synthesis to 84 to 100% of the basal control during the first 2-hr interval, protein synthesis to 73 to 84%, increase in turbidity to 88 to 100%, and DNA synthesis to only 32 to 75%. This results in a gradual increase in the amount of RNA relative to DNA, eventually reaching ratios of 20 to 30, and is followed by a dramatic decrease in the amount of acid-precipitable RNA, between 2 and 5 hr. This is accompanied by cell lysis, as shown by the decrease in turbidity, during the same time interval. Observations with the phase-contrast microscope at 5 hr confirm that lysis has occurred. One observes the presence of extremely long cells (up to ten times the normal length), 95% of which appear empty, and a mixture of intact and lysed protoplast-like bodies. Cells grown in the presence of FU alone, or FU plus thymidine, do not show signs of lysis, but are two to three times their normal length. Cells grown in the presence of a mixture of FU, uridine, and thymidine appear

TABLE 2. *Effect of 5-fluorouracil (FU) on the methionine auxotroph strain M_1**

Additions to basal medium	Time (hr)	Relative increase			No. of viable cells per ml
		Protein	RNA	DNA	
None	0	1.00	1.00	1.00	4.5×10^7
	1	1.49	1.49	2.12	
	2	1.49	1.50	2.31	9.2×10^7
	5				9.9×10^7
FU + uridine	1	1.49	1.83	1.77	
	2	1.57	1.86	1.91	8.7×10^7
	5				1.1×10^8
Methionine	1	2.44	2.74	2.90	
	2	5.21	7.05	5.55	2.2×10^8
	5				3.5×10^9
FU + uridine + methionine	1	1.93	2.69	1.98	
	2	4.76	6.20	2.81	1.5×10^8
	5				6.6×10^4

* Cells growing exponentially on the basal medium with methionine (1.0 μmole/ml) were harvested by centrifugation, washed three times with the basal medium alone, and portions were distributed in growth flasks with the above additions. The following final concentrations were used: FU at 0.1 μmoles/ml, uridine at 0.5 μmoles/ml, and L-methionine at 1.0 μmole/ml. Incubation was at 37 C. At zero time, the cultures contained the following amounts per ml: 40.5 μg of protein, 9.95 μg of RNA, and 1.0 μg of DNA. Viable counts were determined by plating on basal agar supplemented with L-methionine (1.0 μmole/ml).

normal. Although cells begin to die after a lag of approximately 1 hr in the presence of FU and uridine (Fig. 1), cell lysis is usually not detected before 3 hr of incubation.

Because we had previously found that 8-azaguanine is not bactericidal when an amino acid auxotroph is deprived of its required amino acid (Mangalo and Wachsman, 1962), it was decided to determine whether the combination of FU and uridine behaved similarly. The results of an experiment with a methionine auxotroph (M_1) of strain KM are shown in Table 2. It is apparent that FU and uridine are bactericidal only in the presence of methionine, and that their effect on the biosynthesis of macromolecules in strain M_1 is similar to that observed with strain KM. It is clear that DNA synthesis is less sensitive to methionine starvation than is either protein or RNA synthesis. There is an approximate doubling of the viable count during 5 hr of methionine starvation.

Mutant T_2 requires only thymidine to overcome the inhibitory effects of FU (Table 3). It is apparent that FU alone and the combination of FU and uridine are highly bactericidal. The latter conditions also lead to a gradual increase in the amount of RNA relative to DNA, eventually reaching ratios of 20 to 30. This is followed by a period of rapid degradation of RNA (from 2 to 5 hr) and cell lysis (not shown in table). For the first 2 hr in the presence of FU alone, DNA synthesis is inhibited considerably more than either RNA or protein synthesis.

Mutant FU/2 requires only uridine to overcome the inhibitory effects of FU (Table 4). In the presence of FU alone, DNA synthesis is inhibited somewhat more than RNA synthesis. The addition of thymidine to a culture inhibited by FU stimulates DNA synthesis by 5 to 18% over a 5-hr period, but has essentially no effect on protein and RNA synthesis. Both FU alone and the combination of FU and thymidine are almost equally bactericidal.

The effect of FU on a uridine-requiring auxotroph, strain 239, is shown in Table 5. The addition of FU to a uridine-starved culture permits a small (20 to 25%), but reproducible, stimulation in protein synthesis over a 5-hr period. During this period, there is an abrupt cessation of DNA synthesis and a rapid hydrolysis of RNA. It can also be seen that DNA synthesis is less sensitive to uridine starvation on the basal medium than is

TABLE 3. *Effect of 5-fluorouracil (FU) on strain T_2**

Additions to basal medium	Time (hr)	Relative increase			No. of viable cells per ml
		Protein	RNA	DNA	
FU	0	1.00	1.00	1.00	6.1×10^7
	1	2.01	1.71	1.13	
	2	3.07	1.88	1.09	1.2×10^6
	3	3.16	1.11	1.10	
	5	2.97	0.59	0.97	1.4×10^3
FU + uridine	1	2.04	1.74	1.15	
	2	3.49	2.53	1.36	1.7×10^7
	3	4.56	2.16	1.29	
	5	4.15	0.93	1.24	7.9×10^3
FU + thymidine	1	2.09	1.86	2.03	
	2	3.82	3.10	3.38	3.4×10^8
	3	6.55	4.58	4.93	
	5	15.7	7.52	8.68	8.2×10^8

* Portions of an exponentially growing culture on the basal medium were supplemented with the above compounds at a final concentration of 0.5 μmoles of each per ml. Incubation was at 37 C. At zero time, the cultures contained the following amounts per ml: 79.2 μg of protein, 31.7 μg of RNA, and 2.26 μg of DNA. Samples were removed and plated for viable count as described in Materials and Methods.

TABLE 4. *Effect of 5-fluorouracil (FU) on strain FU/2**

Additions to basal medium	Time (hr)	Relative increase			No. of viable cells per ml
		Protein	RNA	DNA	
FU	0	1.00	1.00	1.00	6.5×10^7
	0.5	1.48	1.13	1.09	
	1	1.83	1.22	1.15	
	2	1.98	1.39	1.18	1.6×10^7
	5	2.53	1.38	1.18	3.5×10^6
FU + uridine	0.5	1.65	1.73	1.81	
	1	2.63	2.82	2.53	
	2	6.50	6.28	6.07	5.6×10^8
	5	20.3	11.2	15.0	2.8×10^9
FU + thymidine	0.5	1.62	1.18	1.15	
	1	1.66	1.19	1.27	
	2	1.82	1.37	1.35	3.3×10^7
	5	2.05	1.42	1.40	2.3×10^6

* Portions of an exponentially growing culture on the basal medium were supplemented with the above compounds at a final concentration of 0.5 μmoles of each per ml. Incubation was at 37 C. At zero time, the cultures contained the following amounts per ml: 80.8 μg of protein, 24.4 μg of RNA, and 1.71 μg of DNA. Samples were removed and plated for viable count as described in Materials and Methods.

either protein or RNA synthesis. The addition of FU to a uridine-containing culture permits substantial RNA and protein synthesis for the first 2 hr. This is followed by a decrease in acid-precipitable RNA and cell lysis (not shown in table). The combination of FU and uridine is more bactericidal than is FU alone, over a 5-hr period.

FU, F-uridine, and FUDR were compared at suboptimal levels (0.02 μmoles/ml) for their growth-inhibitory properties. Based on the viable

TABLE 5. *Effect of 5-fluorouracil (FU) on strain 239, a uridine-requiring auxotroph of KM**

Additions to basal medium	Time (hr)	Relative increase			No. of viable cells per ml
		Protein	RNA	DNA	
None	0	1.00	1.00	1.00	3.7×10^7
	0.5	1.27	1.05	1.22	
	1	1.18	1.04	1.26	
	2	1.22	1.04	1.41	1.1×10^8
	5	1.24	0.98	1.55	1.2×10^8
Uridine	0.5	1.34	1.79	1.53	
	1	2.25	2.47	2.13	
	2	5.49	6.19	4.56	4.6×10^8
	5	15.5	8.80	10.5	1.3×10^9
FU	0.5	1.17	0.97	1.02	
	1	1.25	0.86	1.05	
	2	1.39	0.74	0.95	5.3×10^6
	5	1.53	0.28	1.08	1.1×10^6
FU + uridine	0.5	1.52	1.70	1.15	
	1	2.11	2.35	1.17	
	2	4.56	4.12	1.37	4.3×10^7
	5	3.15	2.72	1.94	1.7×10^5

* Cells growing exponentially on a basal medium with uridine (0.5 μmoles/ml) were harvested by centrifugation, washed three times with the basal medium alone, and portions were distributed in growth flasks with the above additions (final concentration of each compound of 0.5 μmoles/ml). Incubation was at 37 C. At zero time, the cultures contained the following amounts per ml: 87.6 μg of protein, 25.7 μg of RNA, and 2.12 μg of DNA. Viable counts were determined on basal agar supplemented with uridine (0.5 μmoles/ml).

count over a 5-hr period, FUDR was five to ten times more inhibitory for strain KM than were the other analogues. With strains FU/2 and T_2, all three analogues were approximately equally inhibitory.

DISCUSSION

It is clear that uridine enhances the bactericidal effect of FU on strain KM. Strain T_2 growing in the presence of FU alone or with FU and uridine also dies rapidly. These conditions are reminiscent of thymineless death in *E. coli* 15_{T-} (Barner and Cohen, 1957), i.e., killing under conditions where DNA synthesis is severely restricted, whereas RNA and protein synthesis occur at substantial rates. Under optimal conditions, the above two strains were repeatedly observed to undergo a 4- to 5-decade kill within a 5-hr period. Tomasz and Borek (1960) showed that 90 to 95% of the cells of *E. coli* K-12 were killed in the presence of FU, whereas the viable count of *B. subtilis* exposed to FUDR and uracil was reported to decrease to 1% or less within a 4-hr period (Mennigmann and Szybalski, 1962). Cohen et al. (1958) had previously reported that FUDR induces thymineless death in *E. coli*.

The experiment with strain M_1 shows that the combination of FU and uridine is lethal only in the presence of the required amino acid. This is in agreement with the finding of Tomasz and Borek (1960) that FU is only bactericidal for growing cells, and resembles the conditions necessary for killing by 8-azaguanine (Mangalo and Wachsman, 1962). The results show that methionine starvation is not bactericidal over a 5-hr period, and that DNA synthesis, although inhibited, is less sensitive to starvation than is either RNA or protein synthesis. The latter observation is in agreement with the effect of amino acid starvation on *Lactobacillus acidophilus* (Okazaki and Okazaki, 1959) and the requirement of amino acids for DNA synthesis in stringent strains of *E. coli* (Kellenberger, Lark, and Bolle, 1962).

Strain KM requires both uridine and thymidine to overcome the inhibitory effects of FU. Because strain T_2 requires only thymidine to overcome FU

inhibition, it is conceivable that T_2 cannot metabolize FU to a derivative necessary for the inhibition of RNA synthesis. Likewise, strain FU/2 may have lost the ability to convert FU to a derivative (fluorodeoxyuridylic acid) essential for the inhibition of DNA synthesis. However, the possibility that partial resistance is due to the formation of enzymes with reduced affinities for the active derivatives of FU cannot be excluded. The observation that, with either strain T_2 or strain FU/2, FU, F-uridine, and FUDR are equally inhibitory could be a consequence of rapid enzymatic interconversions between the three analogues. White and Nichol (1963) recently reported the isolation of similar mutants from *Pediococcus cerevisiae*.

The effect of FU, with and without the addition of uracil and (or) thymine derivatives, on the synthesis of macromolecules in strain KM, is similar to that observed in *E. coli* B (Horowitz et al., 1960). The latter investigators also found that FU will partially replace uracil for a uracil-requiring auxotroph of *E. coli*, permitting a two-fold increase in protein. With the uridine-requiring auxotroph of *B. megaterium* (no. 239), FU only permits a 20% increase in protein. As with the comparable auxotroph of *E. coli*, DNA synthesis by strain 239 is less sensitive to uridine starvation than is either RNA or protein synthesis.

ACKNOWLEDGMENT

This investigation was supported by research grants AI-01728 and AI-04840 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

LITERATURE CITED

ASHWELL, G. 1957. Colorimetric analysis of sugars, p. 73–105. *In* S. P. Colowick and N. O. Kaplan [ed.], Methods in enzymology, vol. 3. Academic Press, Inc., New York.

BARNER, H. D., AND S. S. COHEN. 1957. The isolation and properties of amino acid requiring mutants of a thymineless bacterium. J. Bacteriol. **74**:350–355.

BURTON, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. **62**:315–323.

COHEN, S. S., J. G. FLAKS, H. D. BARNER, M. R. LOEB, AND J. LICHTENSTEIN. 1958. The mode of action of 5-fluorouracil and its derivatives. Proc. Natl. Acad. Sci. U.S. **44**:1004–1012.

DUSCHINSKY, R., E. PLEVEN, AND C. HEIDELBERGER. 1957. The synthesis of 5-fluoropyrimidines. J. Am. Chem. Soc. **79**:4559–4560.

FLAKS, J. G., AND S. S. COHEN. 1959. Virus-induced acquisition of metabolic function. III. Formation and some properties of thymidylate synthetase of bacteriophage-infected *Escherichia coli*. J. Biol. Chem. **234**:2981–2986.

GORDON, M. P., AND M. STAEHELIN. 1959. Studies on the incorporation of 5-fluorouracil into a virus nucleic acid. Biochim. Biophys. Acta **36**:351–361.

HARBERS, E., N. K. CHAUDHURI, AND C. HEIDELBERGER. 1959. Studies on fluorinated pyrimidines. VIII. Further biochemical and metabolic investigations. J. Biol. Chem. **234**:1255–1262.

HEIDELBERGER, C., N. K. CHAUDHURI, P. DANNEBERG, D. MOOREN, L. GRIESBACH, R. DUSCHINSKY, R. J. SCHNITZER, E. PLEVEN, AND J. SCHEINER. 1957. Fluorinated pyrimidines, a new class of tumor-inhibitory compounds. Nature **179**:663–666.

HOROWITZ, J., J. J. SAUKKONEN, AND E. CHARGAFF. 1960. Effects of fluoropyrimidines on the synthesis of bacterial proteins and nucleic acids. J. Biol. Chem. **235**:3266–3272.

KELLENBERGER, E., K. G. LARK, AND A. BOLLE. 1962. Amino acid dependent control of DNA synthesis in bacteria and vegetative phage. Proc. Natl. Acad. Sci. U.S. **48**:1860–1868.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.

MANGALO, R., AND J. T. WACHSMAN. 1962. Effect of 8-azaguanine on growth and viability of *Bacillus megaterium*. J. Bacteriol. **83**:27–34.

MENNIGMANN, H., AND W. SZYBALSKI. 1962. Transforming activity of DNA isolated from bacteria undergoing "thymine-less death." Bacteriol. Proc., p. 43.

OKAZAKI, T., AND R. OKAZAKI. 1959. Studies of deoxyribonucleic acid synthesis and cell growth in the deoxyribose requiring bacteria, *Lactobacillus acidophilus*. II. Deoxyribonucleic acid synthesis in relation to ribonucleic acid and protein synthesis. Biochim. Biophys. Acta **35**:434–445.

ROGERS, H. J., AND H. R. PERKINS. 1960. 5-Fluorouracil and mucopeptide biosynthesis by *Staphylococcus aureus*. Biochem. J. **77**:448–459.

SCHEINER, J., E. KOSTELAK, AND R. DUSCHINSKY. 1957. 5-Fluoropyrimidines as growth inhibitors of micro-organisms. Federation Proc. **16**:242.

SKÖLD, O. 1958. Enzymic ribosidation and ribotidation of 5-fluorouracil by extracts of the Ehrlich-ascites tumor. Biochim. Biophys. Acta **29**:651.

TOMASZ, A., AND E. BOREK. 1960. The mechanism of bacterial fragility produced by 5-fluorouracil: the accumulation of cell wall precursors. Proc. Natl. Acad. Sci. U.S. **46**:324–327.

VAN NOORT, D., AND A. WALLACE. 1963. Inhibition of chlorophyll synthesis by 5-fluorouracil and 5-fluorodeoxyuridine. Biochem. Biophys. Res. Commun. **10**:109–111.

WACHSMAN, J. T., AND R. MANGALO. 1962. Use of 8-azaguanine for the isolation of auxotrophic mutants of *Bacillus megaterium*. J. Bacteriol. **83**:35–37.

WHITE, P. J., AND C. A. NICHOL. 1963. Effects of uracil and thymidine on the development of resistance to 5-fluorouracil in *Pediococcus cerevisiae*. J. Bacteriol. **85**:97–105.