[17] E. KELLENBERGER AND W. ARBER, *Z. Naturforsch.*, 10b (1955) 698.
[18] S. M. LESLEY, R. C. FRENCH AND A. F. GRAHAM, *Can. J. Research, E* 28 (1950) 281.
[19] S. J. SINGER AND A. SIEGAL, *Science*, 112 (1950) 107.
[20] J. L. ONCLEY, *Ann. N.Y. Acad. Sci.*, 41 (1941) 121.
[21] H. A. SCHERAGA AND L. MANDELKERN, *J. Am. Chem. Soc.*, 75 (1953) 179.
[22] R. GANS, *Ann. Physik*, 86 (1928) 628.
[23] T. F. ANDERSON, C. RAPPAPORT AND N. A. MUSCATINE, *Ann. Institute Pasteur*, 84 (1953) 5.
[24] H. K. SCHACHMAN, *Ultracentrifugation in Biochemistry*, Academic Press, New York, 1959, p. 215.

Biochim. Biophys. Acta, 44 (1960) 445–458

PATTERNS OF CELLULAR CONTROLS OPERATING IN BACTERIOPHAGE REPRODUCTION

I. EFFECT OF 5-FLUOROURACIL ON THE MULTIPLICATION OF SEVERAL COLIPHAGES

FRED GOODMAN, JUSSI J. SAUKKONEN* AND ERWIN CHARGAFF
Cell Chemistry Laboratory, Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N.Y. (U.S.A.)

(Received February 24th, 1960)

SUMMARY

The effects of 5-fluorouracil on bacteriophage production in *E. coli*, strain B, have been studied. In the presence of the fluoropyrimidine the phage yields are less than 0.5 % of the control in T2r infection, whereas as much as 8 % of the control yield is obtained in T3 infection. The addition of uracil does not affect the inhibition of T2r multiplication, but restores T3 formation to 40–50 % of normal. Supplementation with thymidine has little effect on T3, but raises the yield of T2r to more than one fifth of the control. Essentially complete restoration of phage reproduction is observed when both uracil and thymidine are present together with fluorouracil.

Orienting experiments with phages T1 and T4r are also described.

INTRODUCTION

The nature of the control mechanisms that guarantee the unaltered reproduction of the various high polymers of the cell is among the most important problems in chemical biology[1]. There are many ways in which the study of this question may be undertaken, just as there must exist many such cellular controls; but it is clear that no direct approach to their description is discernible at present. We have, in the recent past, repeatedly discussed the conceptual basis of the problem[1–3] and attempted to define it through experimental studies[4–6]. Of particular interest, in this connection,

* Present address: State Serum Institute, Helsinki, Finland.

are those chemical agents that, by interfering with the strict coordination of the synthetic abilities of the cell, are able to disconnect the reproduction of one cellular polymer from that of the others. One such "uncoupling" agent, 5-fluorouracil[7], is studied in this and the following communication[8] in its effect on bacteriophage multiplication.

The action of this fluoropyrimidine on the synthesis of protein and nucleic acid in various strains of *E. coli* has been studied previously in this laboratory. In a uracil-requiring mutant, the substitution of fluorouracil for uracil has been shown to inhibit the formation of deoxyribonucleic acid and almost entirely that of ribonucleic acid, but to permit a considerable increase in protein[5]. The synthesis of several constitutive enzymes continued in the presence of the agent, but no formation of inducible enzymes could be observed[5,9]. In *E. coli*, strain B, as well as in the uracil auxotroph in the presence of uracil, 5-fluorouracil inhibited the synthesis of deoxyribonucleic but not of ribonucleic acid; it was even found to be incorporated into the latter in a considerable quantity[10].

The shunt in the cellular controls attendant upon the infection of a cell by a bacteriophage particle or a virus is far-reaching in its consequences. New protein and nucleic acid molecules are produced and there must become operative several enzymes that were either dormant or non-existent in the host. It may be expected that much could be learned about the nature of the normal cellular control mechanisms through a study of the effect of uncoupling agents on the events leading to phage multiplication. In view of the observations on 5-fluorouracil mentioned before, it appeared of interest to compare the effect of this agent on the formation of the even-numbered coliphages of the T series, which require the synthesis of a new nucleic acid component, 5-hydroxymethylcytosine[11], and of the odd-numbered phages, in which no such requirement is apparent.

MATERIALS AND METHODS

5-Fluorouracil was obtained through the courtesy of Dr. J. A. AESCHLIMANN, Hoffmann-La Roche Inc., Nutley, New Jersey. Uracil, 5-bromouracil, thymine and thymidine were commercial preparations of checked purity.

The bacterial strain used in all studies was *E. coli* B which is sensitive to the T phages. The bacteriophage stocks used were T1, T2r, T3 and T4r; they were kindly given us by Prof. F. J. RYAN of this University. The methods used for the preparation of phage stocks of high titer and for the assay of infective particles were adapted from the literature[12].

For each experiment, cells from an overnight culture in synthetic medium[13] or Difco "Penassay" broth were inoculated into tubes containing 5 ml of the particular medium. The tubes were incubated at 37°, with aeration, until an O.D. (read in a Klett-Summerson colorimeter with the use of the 660-mμ-filter) corresponding to $2 \cdot 10^8$ cells per ml was reached. At this time, the various supplements were added to the cultures, as noted in the description of the experiments, and the tubes reincubated. 30 min later, when no further increase in the turbidity of the cultures containing only fluorouracil was noticeable, phage were added. Turbidity readings were taken at intervals until no further changes were noted. The lysates were assayed for plaque-forming units after being kept at 4° overnight.

RESULTS AND DISCUSSION

Inhibition of lysis

The effects of fluorouracil on phage growth in the synthetic medium are shown in Fig. 1. It is apparent that phage production, as measured by the loss of turbidity of infected cultures, is inhibited only in the even-numbered strain tested. It was found that the multiplicity of infection used, affected the shape of the lysis curves. In cultures infected with T3, a decreasing multiplicity of infection resulted only

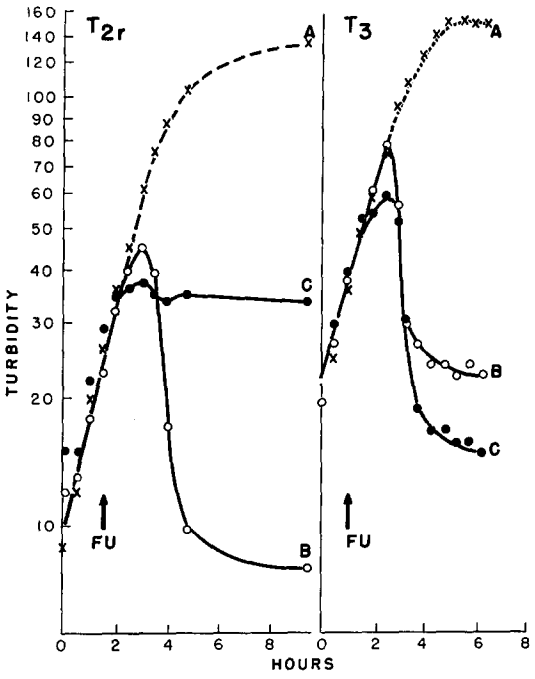

Fig. 1. Effect of 5-fluorouracil on bacterial lysis by phage. FU (50 μg/ml) was added to logarithmically growing cultures of *E. coli* B at the time indicated by arrows. 30 min later, each culture was infected at a ratio of approx. 1 phage particle per 10 bacteria. A, ×—×, control; B, ○—○, infected in absence of 5-fluorouracil; C, ●—●, infected in presence of 5-fluorouracil.

TABLE I

EFFECT OF 5-FLUOROURACIL ON MULTIPLICATION OF T2r

In each experiment 50 μg of 5-fluorouracil/ml of logarithmically growing culture of *E. coli* were employed. 0.5 h later, phage were added to the cultures. At completion of lysis, cells and debris were removed by centrifugation and the supernatant fluids were assayed for plaque-forming units.

Phage particles per ml (as multiples of 10^7)			
Input	*Yield*		*Yield ratio 5-fluorouracil/control*
	Control (without 5-fluorouracil)	*With 5-fluorouracil*	
6	220	1	0.004
60	140	0.7	0.005

in an increase in the time needed for the onset of lysis. On the other hand, infection with a high multiplicity of T2r produced lysis, whereas a low multiplicity of infection resulted in the complete inhibition of lysis. In order to test the production of infective particles under conditions of multiple infection with T2r, samples of a logarithmically growing culture were infected at two different ratios of phage to bacteria, namely, 1:5 and 2:1. Table I indicates that the lysis caused by multiple infection with T2r in the presence of fluorouracil is not accompanied by the release of infective particles.

Since under these conditions the lysis of a cell does not release infective phage, a noticeable drop in turbidity is not to be expected when only a small proportion of the cell population is infected with T2r. If, on the other hand, the first cells to be infected do give rise to infective particles, these will infect the remainder of the population and cause visible lysis regardless of whether new phage particles are or are not synthesized in this second cycle of infection. In all subsequent experiments on lysis, infection was performed at a ratio of one phage per ten bacteria so that less than 10 % of the cells would be infected in the first cycle.

In order to test the effects of the duration of fluorouracil treatment, the substance was added (a) 10 min before the addition of phage, (b) together with the phage, or (c) 12 min after the addition of phage. Fig 2 indicates that the effect of fluorouracil on

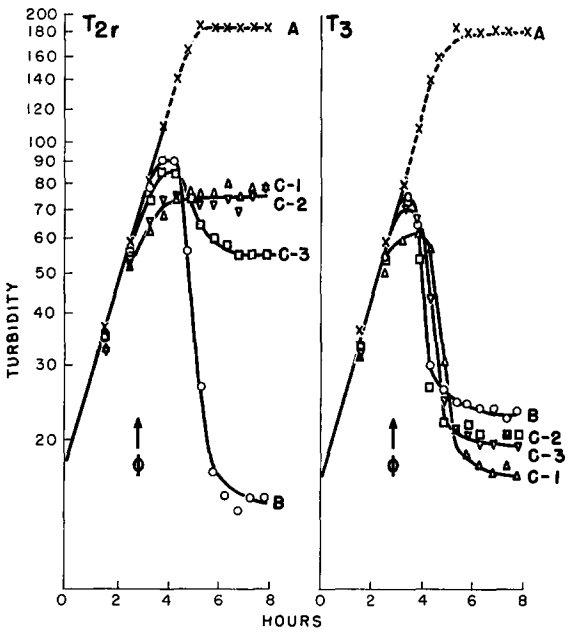

Fig. 2. Effects of duration of treatment with 5-fluorouracil. A, ×—×, control; B, ○—○, infected in absence of 5-fluorouracil; C, 50 μg/ml 5-fluorouracil added: △—△, 10 min before infection; ▽—▽, at time of infection; □—□, 12 min after infection.

lysis induced by T3 is only due to the depression of cell growth. In cultures infected with T2r—although fluorouracil completely inhibits lysis when added simultaneously with phage or before phage addition—some lysis does occur when the compound is added after the phage. This interval—12 min after infection—is about the time of appearance of the first intracellular phage particles[14,15]; and this may be taken to

mean that fluorouracil inhibits the formation of phage rather than its maturation or release from the cell. The difference between the effects on T3 and T2r is not due simply to the shorter latent period of the former, since the addition of fluorouracil to cells as much as 60 min before infection with T3 does not result in the inhibition of lysis.

Reversal of lysis inhibition in T2r infection

One of the effects of 5-fluorouracil appears to be the interference with the enzymic synthesis of thymidylic acid[7,16]. If the block to phage formation were due solely to the inhibition of the production of this nucleotide, supplementation of the medium with thymine or thymidine might be expected to abolish the inhibition. This was not found to be the case. Cells infected with T2r in synthetic medium, in the presence of fluorouracil, required the presence of both thymidine and uracil in order to exhibit lysis (Fig. 3). Several concentrations of uracil up to 20 μg/ml and of thymidine up to 50 μg/ml were tested. The combination of 20 μg of uracil and 40 μg of thymidine/ml was the lowest concentration to support full lysis. Thymidine alone, however, in a concentration as high as 100 μg/ml, was ineffective in overcoming lysis inhibition. It is of interest to note that thymidine alone has no effect on the turbidity of a culture treated with fluorouracil, whereas uracil alone causes an appreciable

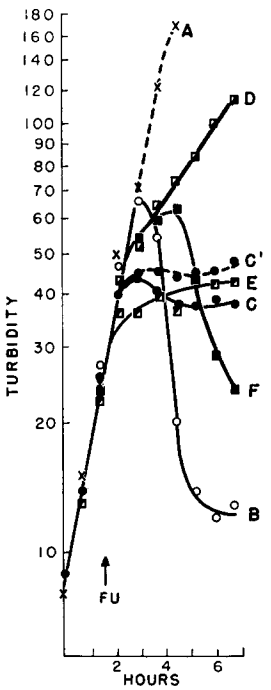

Fig. 3. Reversal of lysis inhibition in T2r infection. A, ×—×, control; B, ○—○, cells infected in absence of pyrimidines; C, ●—●, cells infected in presence of 5-fluorouracil; C′, ●—●, uninfected cells in presence of 5-fluorouracil; D, ◪—◪, cells infected in presence of 5-fluorouracil and uracil; E, ◩—◩, infection in presence of 5-fluorouracil and thymidine; F, ■—■, infection in presence of 5-fluorouracil, uracil and thymidine. Concentrations of pyrimidines per ml: 50 μg 5-fluorouracil; 20 μg uracil; 50 μg thymidine. Time of addition of 5-fluorouracil indicated by arrow; phage added 30 min later.

increase in turbidity (Fig. 3). Thymine can replace thymidine, but slightly higher concentrations of the free pyrimidine are necessary. 5-Bromouracil also showed limited activity as a reversing agent: it could replace thymidine in abolishing the inhibiting action of fluorouracil on lysis and was partially effective in supporting phage growth in the presence of fluorouracil.

The effects of uracil and thymidine on cells treated with fluorouracil appear to be different: both are needed for the full production of phage. We proceeded, therefore to stagger the order and the periods of supplementation. In all cases, actively growing cells received fluorouracil (50 μg/ml) 0.5 h before the addition of phage. The cells then were divided into three series of cultures. To the first series, uracil and thymidine were added simultaneously with fluorouracil or 15, 30, 60 min after the inhibitor. The second series received fluorouracil and thymidine at the same time, and only uracil was added at the noted intervals. In the third series, fluorouracil and uracil were added together, the supplementation with thymidine being staggered. It will be gathered from Fig. 4 that the effects differ. It is clear that in the order of events uracil

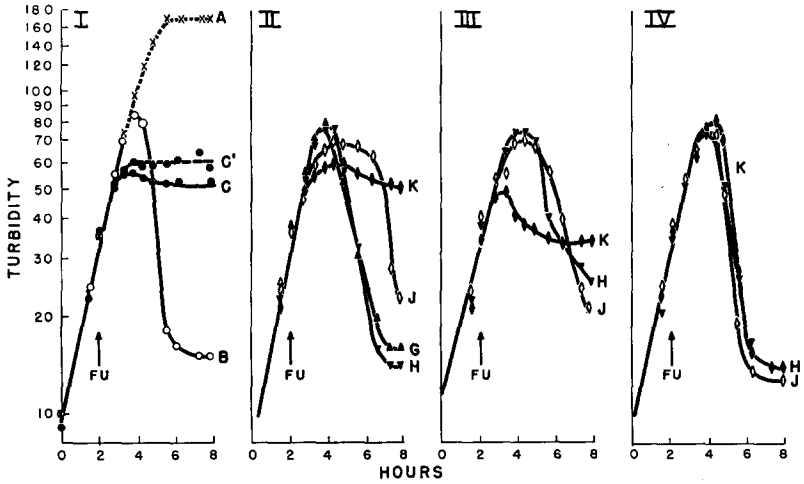

Fig. 4. Effects of delayed addition of supplements on lysis induced by T2r. 50 μg/ml 5-fluorouracil added at time indicated by arrows. Phage added at a ratio of 1 phage per 10 bacteria 30 min after 5-fluorouracil addition. Turbidity curves: I, no supplements present. II, delayed addition of uracil (20 μg/ml) plus thymidine (50 μg/ml). III, delayed addition of uracil to cells treated simultaneously with 5-fluoroucil and thymidine IV, delayed addition of thymidine to cells treated simultaneously with 5-fluorouracil and uracil. A, ×—×, uninfected control, B, ○—○, infection in absence of pyrimidines; C, ●—●, infection in presence of 5-fluorouracil; C', ●—●, uninfected cells in presence of 5-fluorouracil; G, ▲—▲, supplements added simultaneously with 5-fluorouracil; H, ▼—▼, supplements added 15 min after 5-fluorouracil; J, ◇—◇, supplements added 30 min after 5-fluorouracil. K, ◆—◆, supplements added 60 min after 5-fluorouracil.

comes before thymidine. In the presence of uracil, no appreciable delay in lysis is caused by later supplementation with thymidine. In the presence of thymidine, the delayed addition of uracil approximates the effects of the simultaneous addition of both supplements at the various times indicated.

Viability of cells

In order to test the effect of the various supplements in the concentrations used here on the viability of noninfected cells, fluorouracil was added to logarithmically

growing cultures in the synthetic medium simultaneously with either thymidine or uracil or both. Samples of the cultures were removed at intervals, diluted with distilled water and plated on "Penassay" plates. The results are presented in Table II. Fluorouracil has a bactericidal effect which is partially abolished by uracil; thymidine allows some increase in viable cells, whereas both pyrimidines are required for normal increase.

TABLE II

EFFECTS OF SUPPLEMENTS ON VIABILITY OF *E. coli* CELLS

Supplements (μg/ml)			*Viable cells (per ml) × 10^{-6}, after treatment (in minutes)*				
Fluorouracil	*Uracil*	*Thymidine*	*0*	*30*	*60*	*120*	*320*
0	0	0	200	330	530	1400	5400
50	0	0		160	140	26	1.5
50	20	0		270	220	84	54
50	0	50		280	260	230	530
50	20	50		200	360	690	3300

Abolition of fluorouracil effect: quantitative data

In the penultimate section some of the conditions were described that lead to the abolition of the lysis inhibition exerted by fluorouracil. It was of interest to follow the production of infective particles under these conditions. For this purpose lysates obtained by infecting cultures with T2r or T3 in the presence of the pyrimidines were assayed for phage by the agar layer method[12]. The results are presented in Table III.

The quantitative data on the yield of T2r are, with one exception, in good agreement with our previous observations on lysis. It will be seen that fluorouracil inhibits the production of infective particles completely. The addition of uracil and thymidine entirely reverses this inhibition, whereas uracil alone is ineffective. It would, however, not have been predicted, on the basis of our previous observations, that thymidine as the only supplement to the fluorouracil system does have some effect on the inhibition. Under conditions of low phage input, similar to those followed in the experiments in Fig. 3, it restores phage production only to about 3 % of the control value, but with multiple infection the phage yield is about $^1/_5$ of that of the control. The presence of fluorouracil was not found to inhibit the adsorption of phage appreciably, only 1 to 2 % of the phage input remaining unadsorbed after 5 min under all conditions tested. It was also found that the various pyrimidines, alone and in combination, are without effect on the free phage.

Phage T3 is inhibited, but to a much lesser extent than is T2r by fluorouracil. Addition of uracil results in the production of about one-half the normal phage yield, whereas supplementation with both thymidine and uracil abolishes the inhibition almost completely. Thymidine alone appears to have no effect when all cells are infected in the first cycle of infection, but increases the phage yield when only a small proportion of cells are infected originally.

It has been suggested that the inhibitory effect of fluorouracil on *Lactobacillus leichmanii* is reversed non-competitively by thymine or thymidine and competitively by uracil[7]. We tested the effects of varying concentrations of these compounds on

TABLE III

EFFECT OF 5-FLUOROURACIL AND OF REVERSING AGENTS ON PRODUCTION OF PHAGES T1, T2r, T3, AND T4r*

Supplements (μg/ml)			*T1*		*T2r*		*T3*		*T4r* **	
Fluorouracil	*Uracil*	*Thymidine*	*Low input*	*High input*	*Low input*	*High input*	*Low input*	*High input*	*Low input*	*High input*
			Relative phage yield ***							
0	0	0	100 ($2.9 \cdot 10^{10}$)	100 ($1.4 \cdot 10^{10}$)	100 ($2.1 \cdot 10^{10}$)	100 ($1.6 \cdot 10^{10}$)	100 ($1.2 \cdot 10^{10}$)	100 ($1.8 \cdot 10^{10}$)	100 ($3.1 \cdot 10^{11}$)	100 ($8.7 \cdot 10^{10}$)
50	0	0	0.002	0.71	0.011	0.49	1.3	8.3	0.0003	0.054
50	20	0	0.45	1.63	0.013	0.61	49	43	0.0005	0.123
50	0	50	13	6.3	2.8	22	7.3	7.8	0.062	0.78
50	20	50	100	64	156	141	91	96	17	70
			Phage particles per viable cell §							
0	0	0	54	44	40	49	23	55	585	167
50	0	0	0.0041	0.64	0.016	0.49	1.1	9.4	0.0067	0.16
50	20	0	0.59	0.89	0.012	0.36	27	29	0.0071	0.22
50	0	50	14	3.2	2.3	13	3.3	5	0.74	1.3
50	20	50	80	46	91	113	30	87	146	165

* Cell density approximately $2 \cdot 10^8$/ml at the time of addition of supplements. Phage added 30 min later; "low input" less than 1 phage/2 cells; "high input" more than 2 phage/cell.

** 100 μg/ml tryptophan added to permit adsorption.

*** Relative yields represent means of three independent experiments, with the control yields (actual phage titers in parentheses) taken as 100.

§ Titer of phage divided by the number of viable cells at time of infection (see Table II). It was assumed that infection occurs 30 min after supplement addition at high phage input, 60 min at low input.

phage T2r production. The results of one experimental series are shown in Table IV. It will be seen that in the presence of 50 μg/ml of fluorouracil, thymidine as the only supplement, regardless of its quantity, permitted only 2 % of the phage yield of the control. Thymine alone was much less effective than thymidine, but only slightly less so when compared with the nucleoside in the presence of uracil. In systems supplemented with uracil the phage yield increased very noticeably with increasing concentrations of thymidine or thymine. A limited, but definite, effectiveness of 5-bromouracil in overcoming the inhibitory action of the fluoro compound is also evident.

TABLE IV

T2r PRODUCTION IN PRESENCE OF 5-FLUOROURACIL AS A FUNCTION OF DIFFERENT SUPPLEMENT CONCENTRATIONS*

Supplements (μg/ml)					*Phage per ml* ($\times 10^{-7}$)
5-Fuorouracil	*Uracil*	*Thymidine*	*Thymine*	*5-Bromouracil*	
0	0	0	0	0	580
50	0	0	0	0	0.013
50	0	20	0	0	9.0
50	0	50	0	0	12
50	0	100	0	0	11
50	0	0	20	0	0.60
50	0	0	50	0	4.4
50	0	0	0	50	0.64
50	20	20	0	0	79
50	20	50	0	0	930
50	20	0	20	0	66
50	20	0	50	0	530
50	20	0	0	50	61

* All supplements were added 30 min before infection with 1 phage per 10 bacteria.

In an additional series of experiments the competitive reversal by uracil alone at different levels of fluorouracil was examined. At concentrations of 10 or 20 μg/ml of the inhibitor, equal quantities of uracil permitted a partial restoration of phage production (3 % or more of the control yield). At the fluorouracil level employed in most of the present studies, 50 μg/ml, almost no restitution by uracil as the only supplement was observed.

Effect of 5-fluorouracil on other phages

In view of the remarkable difference in the effectiveness of fluorouracil in inhibiting the production of T2r and T3 phages, it was of interest to obtain orienting quantitative data on some other members of the T series. The effects of fluorouracil and of the various supplements on the multiplication of phages T1 and T4r are also included in Table III. Although large quantitative differences in the response of the various phage strains to the supplements are apparent, the findings are consistent qualitatively. Fluorouracil inhibits the production of all the phage strains tested to some extent: most markedly that of the even phages, least of T3. Phage T1 appears to be intermediate. Supplementation with thymidine alone partially reverses the inhibition; the addition of both thymidine and uracil greatly stimulates the production of all phages. The effect of uracil alone differentiates between the odd and the even phages tested. In the case of T2r and T4r there is no stimulatory effect by uracil,

whereas the production of the odd-numbered phages, especially T3, is markedly stimulated by this pyrimidine.

Additional remarks

The order of events taking place during the "eclipse" period of infectivity, when the reorganization of the synthetic apparatus of the host brings about the production of viral components is less well known than some of the events themselves[17]. Among these there appears to be, at any rate in the case of T2r, the synthesis of new proteins and of a small amount of a specific ribonucleic acid. It was, in the light of our previous findings on the effects of 5-fluorouracil on *E. coli*[5,9,10], not without interest to compare the effects of this pyrimidine on the multiplication of some of the even- and odd-numbered T phages.

5-Fluorouracil appears to exert at least two distinguishable effects on the cell: it interferes with the normal synthesis of ribonucleic acid[18] and inhibits the formation of thymidylic acid[16,18]. To take the specific case of *E. coli* B, the host of the phages under consideration here: in the presence of the fluoro compound, the synthesis of deoxyribonucleic acid is blocked completely while that of protein and ribonucleic acid continues[9]. The addition of uracil to the inhibition system results in a stimulation of the formation of the two polymers mentioned last, but not of deoxyribonucleic acid; that of thymine, on the other hand, permits the resumption of deoxyribonucleic acid synthesis to a very limited extent without affecting the synthetic rates of the other compounds. Full supplementation (uracil and thymine) largely overcomes all blocks.

The findings described in the following paper[8] do, in fact, indicate that 5-fluorouracil blocks the synthesis of deoxyribonucleic acid completely in cells infected with T2r+. Uracil is without effect on this inhibition; thymidine permits synthesis, but at a considerably slower than normal rate. When the system containing the fluoro compound is supplemented with both uracil and thymidine the degree of formation of deoxyribonucleic acid equals, or even surpasses, that of the controls.

In view of the great differences between phages T2r and T3 as regards the respective origins and quantities of required precursor material[17], a difference in the effects of 5-fluorouracil was to be expected; and this is borne out by the findings presented here. Different phages are affected differently. The multiplication of phage T2r is paralyzed most efficiently by the fluoro compound; an inhibition that is partially abolished by the simultaneous presence of thymidine, but not at all by uracil alone (Table III). According to the few results available for T4r the trend seems similar, but this phage is even more sensitive to the inhibitor. In contrast, phage T3 is much less affected by fluorouracil and rehabilitated to a large extent by uracil alone, but not by thymidine. In its response to inhibitor and reversal agents phage T1 is more similar to the even-numbered phages than to T3. Full supplementation with both uracil and thymidine largely abolishes the inhibition of all phages.

If one were to distinguish between a "uracil step" and a "thymidine step" in the pattern of the reversal of the fluorouracil effects by pyrimidine supplements, the results summarized in Table III and in Fig. 4, taken together with the findings presented in the following paper[8], would seem to indicate that the first step is concerned with the synthesis of "initial protein" (including newly arising enzymes), the second with that of deoxyribonucleic acid. In phages requiring the massive formation of the latter compound (*e.g.*, T2r and T4) uracil is ineffective, presumably

because it is unable to reinstate the capacity of synthesizing thymidylic acid. On the other hand, thymidine as the only supplement exhibits a limited effectiveness in the same phages which is perhaps due to a more decisive inhibition, by fluorouracil, of the methylation step leading to the thymine component of deoxyribonucleic acid than of the metabolism of ribonucleic acid. The depression of the production of phage T3, however, appears to concern primarily the "uracil step".

It is not inconceivable that bacteria infected with T2r phage in the presence of fluorouracil and uracil are able to produce some phage protein. Preliminary tests for the presence of phage "ghosts"[19] in lysates of such cells have failed to provide evidence of the formation of killing particles. We are, however, continuing these experiments, including the use of immunological tests for the production of phage protein.

ACKNOWLEDGEMENTS

This work has been supported by research grants from the National Institutes of Health, United States Public Health Service, and from the National Science Foundation. J. S. S. was aided by the S. Juselius Foundation and the State Science Committee, Helsinki, Finland.

REFERENCES

1 E. CHARGAFF, *Chemical Aspects of Biological Specificity, The Jesup Lectures* 1959, Columbia University Press, in preparation.
2 E. CHARGAFF, in *The Origin of Life on the Earth.* (I.U.B. Symposium Series, Vol. I), Pergamon Press, London, 1959, p. 297.
3 E. CHARGAFF, *Transactions, 4th International Congress of Biochemistry*, Vol. XIV, Pergamon Press, London, 1959, p. 21.
4 A. LOMBARD AND E. CHARGAFF, *Biochim. Biophys. Acta*, 25 (1957) 549.
5 J. HOROWITZ, J. J. SAUKKONEN AND E. CHARGAFF, *Biochim. Biophys. Acta*, 29 (1958) 222.
6 J. HOROWITZ, A. LOMBARD AND E. CHARGAFF, *J. Biol. Chem.*, 233 (1958) 1517.
7 C. HEIDELBERGER, N. K. CHAUDHURI, P. DANNEBERG, D. MOOREN, L. GRIESBACH, R. DUSCHINSKY, R. J. SCHNITZER, E. PLEVEN AND J. SCHEINER, *Nature*, 179 (1957) 663.
8 J. J. SAUKKONEN, F. GOODMAN AND E. CHARGAFF, *Biochim. Biophys. Acta*, 44 (1960) 469.
9 J. HOROWITZ, J. J. SAUKKONEN AND E. CHARGAFF, in preparation.
10 J. HOROWITZ AND E. CHARGAFF, *Nature*, 184 (1959) 1213.
11 G. R. WYATT AND S. S. COHEN, *Nature*, 170 (1952) 1072.
12 M. H. ADAMS, *Bacteriophages*, Interscience Publishers, Inc., New York, 1959, p. 443.
13 B. D. DAVIS AND E. S. MINGIOLI, *J. Bacteriol.*, 60 (1950) 17.
14 A. H. DOERMANN, *J. Gen. Physiol.*, 35 (1952) 645.
15 J. I. TOMIZAWA AND S. SUNAKAWA, *J. Gen. Physiol.*, 39 (1956) 553.
16 S. S. COHEN, J. G. FLAKS, H. D. BARNER, M. R. LOEB AND J. LICHTENSTEIN, *Proc. Natl. Acad. Sci.*, 44 (1958) 1004.
17 G. S. STENT, in F. M. BURNET AND W. M. STANLEY, *The Viruses*, Vol. 2, Academic Press, New York and London, 1959, p. 237.
18 P. B. DANNEBERG, B. J. MONTAG AND C. HEIDELBERGER, *Cancer Research*, 18 (1958) 329.
19 R. M. HERRIOTT AND J. L. BARLOW, *J. Gen. Physiol.*, 41 (1957) 307.