THE INDUCTION OF MUTANTS OF BACTERIOPHAGE T2 BY 5-BROMOURACIL

IV. KINETICS OF BROMOURACIL-INDUCED MUTAGENESIS*

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SUMMARY

A kinetic study has been made of the effects of 5-bromouracil deoxyriboside (BrUDR) on T2 development in a sulfanilamide-containing medium. As tested by the u.v. light sensitivity of the intracellularly developing phage and by the time of appearance of the first intracellular mature phages, it was found that sulfanilamide and BrUDR did not change the pattern of phage development, but slowed it down slightly. Sulfanilamide caused lysis inhibition of the r_2 phages in this medium. BrUDR did not begin to be effective as a mutagen until the time expected for commencement of phage DNA synthesis, but the mutagenic action of BrUDR could be exerted at any time thereafter as long as phage DNA continued to be synthesized. Counteraction of free BrUDR by thymidine at 15 min after infection did not halt the continued production of mutants. It is proposed that mutations are induced both in the process of the introduction of BrU into phage DNA and during the replication of DNA containing BrU. Two mechanisms by which BrU might induce the appearance of altered base sequences in phage DNA are outlined.

INTRODUCTION

The work presented in the preceding papers[1-3] points to a direct connection between bromouracil-induced mutagenesis and bacteriophage deoxyribonucleic acid (DNA) metabolism and composition. These studies were limited in that only the progeny phage and other final products were examined; that is, the results of certain treatments or conditions applied at the time of infection were examined only at the end of the phage developmental cycle. In the present communication, an attempt has been made to obtain information on the mechanism of bromouracil mutagenesis by a series of kinetic investigations concerned with the determination of when a bromouracil compound acts during phage development.

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The schedule of events occurring in T2-infected *Escherichia coli* B is now quite well defined. Although the subject of phage development has been treated at length in several recent reviews[4,5] the following brief discussion will suffice to permit correlation of the time of the establishment of the induced mutations with the development of phage. T2 infects *E. coli* B by first attaching by its tail to the host bacterium and dissolving a small region of the bacterial cell wall[6]. Then the phage DNA passes into the cell, while the phage protein coat remains on the surface of the bacterium and has no further function in phage reproduction[7]. Immediately following phage DNA entry, a reorganization of the metabolism of the bacterial host takes place. The synthesis of ribonucleic acid is reduced to 2 to 3% of that in the uninfected bacterium[8], total protein synthesis continues without interruption[9], the synthesis of bacterial DNA is at once stopped, and phage DNA synthesis starts at about 7 min[10]. Only phage DNA is synthesized in infected bacteria[11], and it continues to be synthesized throughout the latent period and is always present in excess of the number of mature phage particles formed[12]. During the first 12 min after infection, the phage has lost its identity as an infectious unit[13]. After 12 min, however, mature infective phages begin to make their appearance within the cell and continue to increase in number until the infected bacterium lyses at the end of the latent period[14].

MATERIAL AND METHODS

The materials and methods described in the preceding publications[2,3] were applied in the present work. 5-bromouracil deoxyriboside (BrUDR) was used as a mutagenic agent, since it caused many more mutants to appear than did 5-bromouracil[3]. The infective phage yield is the number of phage particles capable of forming plaques produced per infected bacterium, and the percentage of plaque-type mutants is the number of mutant plaques of all types observed × 100 divided by the total number of plaques.

RESULTS

Determination of the time course of T2 development in the presence of sulfanilamide and BrUDR

Since the correlation of the time course of the mutagenic action of BrUDR with other events in the infected bacterium was the principal objective of the present work, it was necessary to determine whether BrUDR seriously altered the schedule of phage development. Release of phages from infected bacteria with chloroform at various times after infection showed that in the presence of BrUDR the first mature phages appeared at about 15 min. This result suggests that phage development in the presence of sulfanilamide (sulfa) and BrUDR is slightly slower than in the absence of these compounds. (In the latter case, the first intracellular phage appear at about 12 min.) This conclusion is borne out by the experiments described below.

A second method of measurement of the time of appearance of the first intracellular phage was performed by plating with streptomycin resistant bacteria on streptomycin plates. Streptomycin stops phage development within 2 or 3 min after its addition, but the infected bacteria lyse at the normal time[15]. Therefore, until the appearance of the first mature phages, only the unadsorbed phages will form plaques

on the streptomycin plates. The results of these platings are presented in Table I. It can be seen that the number of unadsorbed phages decreased slightly between 0 and 10 min, but by 15 min the plaque titer had begun to rise, indicating the appearance of mature phage before 18 min (15 min plus 3 min for the delay needed for streptomycin action). By extrapolation, one finds that the first mature phages made their appearance at about 15 min after infection. Under normal conditions, the first intracellular mature phages begin to appear at 12 to 13 min[13]. These results demonstrate once more that the time course of T2 development in the presence of sulfa and BrUDR was slightly delayed.

TABLE I

TIME OF APPEARANCE OF INTRACELLULAR PHAGE IN THE PRESENCE OF SULFA AND BrUDR

E. coli B was grown for four generations in 10 ml of VM medium[3] to a density of 10^8 cells per ml, then centrifuged, washed and resuspended in 1 ml of M-9 medium without glucose. T2r_2 was added at a multiplicity of 0.02 phages per bacterium, and adsorption allowed to proceed for 5 min at 37°. The infected culture was then diluted by the addition of 9 ml of the original growth medium now supplemented with 50 μg/ml BrUDR, and the time of dilution taken to be time 0 (90% of the phages had been absorbed by this time). At 0, 5, 10, 15, and 20 min after infection (and after the addition of BrUDR), aliquots of the infected bacteria were diluted and plated with a T2-sensitive, streptomycin resistant strain of *E. coli* B on agar containing 2 mg/ml of streptomycin.

Time after infection and addition of BrUDR in minutes	*Results of the streptomycin platings*		
	Plaque titer	*% Infected bacteria containing mature phage**	*Presence of plaque-type mutants*
0	$4.8 \cdot 10^3$	0	—
5	$3.0 \cdot 10^3$	0	—
10	$2.7 \cdot 10^3$	0	—
15	$5.6 \cdot 10^3$	15	+
20	$15.0 \cdot 10^3$	65	+

* = 100 × plaque titer at any time minus the number of unadsorbed phage divided by the number of originally infected bacteria per ml.

Table I also indicates the presence or absence of plaque-type mutants. Their exact proportion could not be determined because of the poor plating conditions obtained when using the streptomycin technique. Nevertheless, mutant plaques appeared only on the 15- and 20-min plates, those which exhibited plaques formed by infected bacteria containing mature phage. Therefore, mutants made their appearance at the same time as the first few intracellular mature phages.

To compare the time course of T2 development in the presence of sulfa and BrUDR with that occurring in infected bacteria in normal media, the sensitivity of infected bacteria to u.v. light at different times after infection was determined. In 1947, LURIA AND LATARJET[16] demonstrated that the u.v. sensitivity of the infected bacterial cell at different stages in the phage life cycle followed a very specific pattern and suggested that this pattern reflected the evolution of the infectious process. The technical improvements devised by BENZER[17] and by SYMONDS[15] were employed in the present experiments. The family of curves obtained (Fig. 1) closely resembles that obtained under normal conditions. At 0 min the resistance of the infective centers to u.v. light was slightly greater than that of free phage. The resistance to u.v. light increased slowly between 0 and 5 min, then very rapidly between 5 and 10

min, and finally reached a peak at 15 min after infection. At 15 min the resistance to u.v. light was about 30 times greater than that at 0 min. After 15 min, the resistance of the infective centers to u.v. light decreased. While the shapes of the curves presented in Fig. 1 and the extent of increase in u.v. light resistance were identical to those obtained normally, the results show that the development of the resistance of infected bacteria to u.v. light was slowed down by sulfa and BrUDR. Normally the peak of u.v. light resistance is obtained at 10 min, whereas in the present experiment, it occurred at about 15 min. Therefore, as determined by sensitivity to u.v. light, the development of T2 in the presence of sulfa and BrUDR folowed the normal pattern but was slightly retarded.

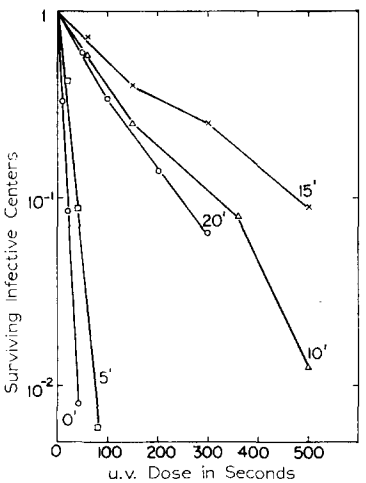

Fig. 1. Inactivation by u.v. light of infectious centers of $T2r_2$ in sulfa and BrUDR. The procedures for growth and infection of the bacteria were identical to that of the experiment of Table I. At 0, 5, 10, 15 and 20 min an aliquot was withdrawn, diluted 100-fold into ice-cold minimal salts buffer, and kept on ice until all of the samples for irradiation had been collected. 3 ml of each of these dilutions was placed on a separate watch glass over cracked ice and irradiated with 350 ergs/cm²/sec of u.v. light from a 0.35 amp. Rad-i-air lamp at a distance of 82 cm for the lengths of time indicated in Fig. 1. After each dose of u.v. light, aliquots were withdrawn from the 3 ml and diluted and plated on *E. coli* B for numbers of surviving infective centers. The number on each curve gives the time in minutes between infection and dilution into cold buffer. Ordinate: fraction of infected bacteria which retained the ability to liberate phage.

Kinetics of phage and phage mutant production in the continuous presence of BrUDR

Since the time course of T2 development in the presence of sulfa and BrUDR follows the normal pattern at a slower rate, it is possible to determine at what stage during phage infection BrUDR exerts its mutagenic effects. First, studies were made of the phage and phage mutant production as a function of time by bacteria infected and allowed to remain in the presence of BrUDR. The results of the platings on *E. coli* B are plotted in Fig. 2 as a function of time after infection. The infective phage production in the chloroform treated aliquots proceeded at a nearly constant rate during the 180 min of the experiment. More striking, the number of mutants increased even more rapidly, so that the *percentage* of mutants actually rose with increasing time of exposure to BrUDR. At first this value rose very steeply, then more slowly,

reaching a final value of 9 % at 180 min. Apparently few phages were released through spontaneous lysis (centrifuged samples), but among these the percentage of mutants also increased with time after infection and BrUDR addition. The results of this experiment show first that the infection of *E. coli* B with a high multiplicity of $T2r_2$ in the presence of sulfa and BrUDR maintained the bacteria in a lysis inhibited state, a phenomenon observed normally only with r^+ phages[18]; other experiments showed that BrUDR was not needed to produce lysis inhibition. Second, in this condition, the infected bacteria synthesized infective phages at a nearly constant rate; and third, the continued presence of BrUDR during infection caused the induction of a constantly increasing *proportion* of mutants. It would, therefore, appear that the mutagenic action of BrUDR continued to be expressed throughout the time that phages were being synthesized.

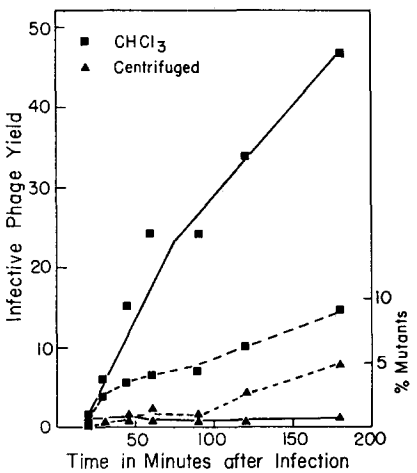

Fig. 2. Production of phage and phage mutants by bacteria in the presence of sulfa and BrUDR. *E. coli* B was grown in VM medium, infected with a multiplicity of 6 $T2r_2$ phages per bacterium, and 50 μg/ml BrUDR added immediately afterwards. At various times after infection, an aliquot of the infection mixture was lysed with chloroform to recover total mature phages, while another aliquot was centrifuged for 5 min at low speeds to remove unlysed bacteria and the supernatant fluid was analysed for phages released through spontaneous lysis. ■ = total mature infective phages; ▲ = infective phage released through spontaneous lysis. ——— = infective phage yield; – – – = percentage of plaque-type mutants.

To provide further support for the above conclusion, an experiment was performed to determine whether BrUDR could still induce the appearance of mutants if added at a time (40 min after infection) when the phage-producing mechanism was already established. The results obtained are plotted in Fig. 3. As was observed previously, in the 0′ sample, phages were produced at a linear rate, and the percentage of mutants rose with increasing time of exposure to BrUDR. In the culture to which BrUDR had been added at 40 min the rate of infective phage production, at first much greater than in the 0′ sample, was later diminished; but mutants appeared although only after a lag of a least 50 min. In the culture to which BrUDR was added at 40 min 5 % of the phages produced between 90 and 180 min were mutants. These data show that BrUDR must not cause mutant production only by modifying the initial steps of infection.

Effect of the removal of BrUDR at various times after infection

The results of the two preceding experiments suggest that the mutagenic action of BrUDR is not confined to any one period of phage development and can still be exerted during the late stages of phage reproduction. However, because the major reorganization of the metabolism of the infected bacteria takes place during the first few minutes after infection, it was of interest to determine whether the initial time of establishment of BrU mutagenesis could be correlated with a particular time during the initial stages of phage development. Experiments were performed to determine the effects of a brief initial exposure to BrUDR.

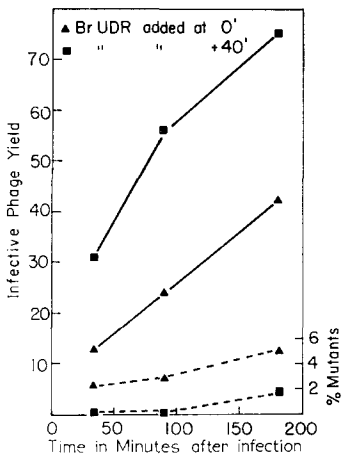

Fig. 3. Effects of the addition of BrUDR at infection or at 40 min afterwards. Two cultures of *E. coli* B were grown in VM medium and infected with 6 T2r_2 per bacterium. To one culture 50 μg/ml BrUDR was added immediately after infection (0'), while the other received 50 μg/ml BrUDR 40 min later (+ 40'). Aliquots were withdrawn from each culture at 35, 90, and 180 min to be lysed with chloroform. ——, infective phage yield; – – –, percentage of plaque-type mutants.

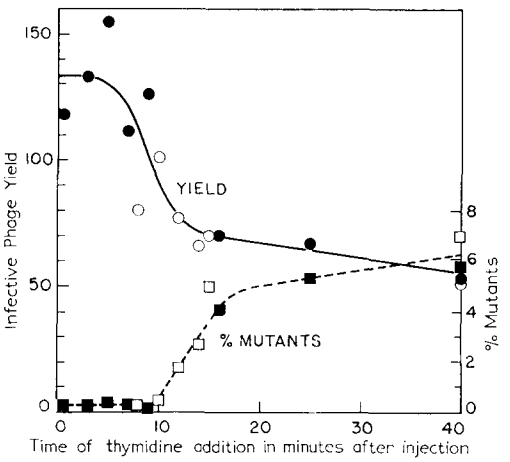

Fig. 4. Effect of the addition of BrUDR and of thymidine at various times after infection. *E. coli* B was grown in VM medium, infected at time 0 with 6 T2r_2 per bacterium, and 50 μg/ml BrUDR added immediately to all of the samples. Then at the times indicated in Fig. 4, 50 μg/ml thymidine was added to individual tubes and the infected bacteria in every sample lysed with chloroform three hours after infection. ——, infective phage yield; – – –, percentage of plaque-type mutants. Solid and open symbols distinguish the two experiments.

It was shown in the preceding paper[3] that thymidine, added simultaneously with BrUDR at the time of phage infection, had the property of specifically preventing the action of BrUDR; and it was found that the addition of this natural antagonist was a more effective way to stop BrUDR action than was simple dilution. The prevention of BrUDR action was studied as a function of time of thymidine addition. The results of two such experiments, plotted in Fig. 4 as a function of the time of addition of thymidine, represent the infective phage yield and the percentage of mutants obtained at the end of 3 h. If thymidine was added prior to 10 min after infection (and after the addition of BrUDR), the percentage of mutants obtained at 3 h remained at a constant low value, while the phage yields were quite high. This percentage of mutants and the phage yields were identical to those obtained in the

sulfa control (not indicated in the Figure). Initially, therefore, the addition of thymidine effectively blocked the mutagenic and inhibitory action of BrUDR. But after this period the addition of thymidine became progressively less effective; the percentage of mutants began to rise and the phage yields to drop. The results also indicate that BrUDR is more effective during the period of 10 to 20 min after infection than it is later on.

It would appear, therefore, that BrUDR was effective as a mutagen only from about 10 min after infection on. This time coincides in the sulfa and BrUDR medium with the expected time of the start of phage DNA synthesis in sulfa and BrUDR

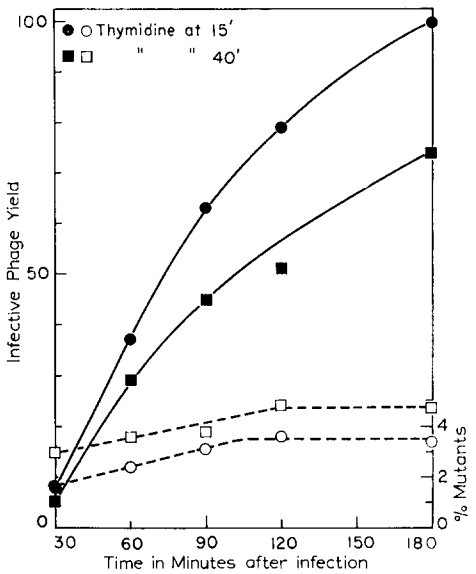

Fig. 5. Effects of an initial exposure to BrUDR. *E. coli* B was grown in VM medium, infected with 6 $T2r_2$ per bacterium, and 50 μg/ml BrUDR was added immediately afterwards. Then in one sample, 50 μg/ml of thymidine was added at 15 min (TD 15) and in another at 40 min (TD 40). At various times, aliquots were removed from both samples, lysed with chloroform, and diluted and plated. (The procedure used in this experiment was identical to that of Fig. 2 except for the addition in the present case of thymidine.) ——, infective phage yield; – – –, percentage of plaque-type mutants.

medium, in which phage development is slowed down by about 3 min. The coincidence between the time of the effectiveness of BrUDR as a mutagen and the time of phage DNA synthesis suggests a requirement of DNA synthesis for the mutational event induced by BrUDR.

The technique utilized in the preceding experiment also presents an opportunity of learning when the mutations induced by BrUDR take place. As seen from Fig. 4, when thymidine was added as early as 15 min after infection (and after the addition of BrUDR) a high proportion (3.5 %) of mutants was observed when the bacteria were caused to lyse almost 3 h later. Does this percentage of mutants represent the extent of BrUDR mutagenic action between 10 and 15 min alone, or can changes produced during this time lead to new mutational events between 15 min and 3 h? In order to distinguish between these two possibilities, the rates of phage and phage mutant production were determined when BrUDR was added at the time of infection and thymidine subsequently at 15 (TD 15) or 40 min (TD 40).

The results plotted as infective phage yield and percentage of mutants as a function of time after infection are presented in Fig. 5. At all times the phage yield was higher and the percentage of mutants lower in the TD 15 sample than in the TD 40 sample. The total number of mutants increased at least 15-fold between 30

and 180 min in both cases. The percentage of mutants in both samples also rose for at least 90 min before attaining a final value of 3.5 and 5 % for the TD 15 and the TD 40 samples, respectively, and was always higher than that existing at the time of thymidine addition.

In considering the interpretation of these data the possibility should be considered that thymidine might not immediately stop the action of free BrUDR. This is unlikely because at 30 min after infection, the TD 15 sample contained a lower percentage of mutants and a slightly higher phage yield than did the TD 40 sample to which thymidine had not yet been added. Although this does not prove that thymidine, immediately upon its addition, blocks BrUDR action, it does negate the possibility of a long delay in thymidine action, since an effect of thymidine was noted in the first aliquots taken after its addition.

The results of Fig. 5, at least those of the TD 15 sample, demonstrate that many new mutational events had taken place after the addition of thymidine, as distinct from those which had occurred when free BrUDR was available for phage DNA synthesis; *i.e.*, between approximately 10 and 15 min. Initially BrUDR was undoubtedly incorporated into the newly synthesized phage DNA, and the continued increase in the number of mutants after the addition of thymidine at 15 min could be due to new mutational events which arose during the replication of the BrU containing DNA.

DISCUSSION

The following model of BrU action is consistent with the results described in this series of papers. One assumes that vegetative phages reproduce exponentially[19] and that replicating units of vegatative phages consist of DNA. Phage DNA in the process of being synthesized can incorporate BrU, and these BrU-containing DNA molecules enter the pool of phage precursor DNA. A precursor can replicate to give rise to further precursors until it is incorporated into a mature phage. Hereditary alterations in the DNA structure can occasionally arise during the introduction of BrU into DNA, and these "mutations" can be replicated upon replication of the precursor DNA. Furthermore, replication of non-mutant DNA (containing BrU) can also create hereditary alterations by the mechanism indicated below. Some of the hereditary alterations in DNA structure are expressed as mutations in succeeding phage generations.

This scheme is consistent with the following observations, made in the present series of papers: (a) BrU incorporation is always accompanied by the appearance of mutants[3]. (b) BrU mutagenesis is prevented by thymidine and involves DNA synthesis[3]. (c) BrUDR mutagenesis commences at the same time as does phage DNA synthesis. (d) BrUDR is mutagenic in the absence of protein synthesis[2,20]. (e) The phages first formed include mutants. (f) A single bacterium releases both mutant and non-mutant phages[1]. (g) When BrUDR is present throughout the growth cycle, the number of mutants increases faster than the total number of phages; and therefore the percentage of mutants continually increases. (h) When the BrUDR is displaced by thymidine soon after mutagenesis is initiated, mutants continue to appear as an increasing percentage of the phages. (i) When BrUDR is added after some mature phages have already been formed, the percentage of mutants in the phages completed

after that time gradually increases and eventually reaches a value similar to that found when BrUDR is added initially.

The validity of other schemes of mutagenesis should be considered in the light of the above observations.

According to the above scheme, the appearance of an increasing percentage of mutants with time is attributed to changes occurring on replication of the non-mutated, BrU-containing DNA. Alternative explanations can be advanced which depend on gradual enrichment of the phage precursor pool with mutated DNA, as the non-mutated DNA is withdrawn. (That the composition of the precursor pool can change is indicated by the experiment in which addition of BrUDR at 40 min causes mutants to appear.) One can imagine, for example, that since the first phages formed contain DNA made from DNA of the bacteria[21], fewer mutants would be found in the first phages.

Similarly, in the experiments in which BrUDR was effectively present only for an initial period (Fig. 5) and yet mutants continued to appear as an increasing percentage of the phages, an explanation can be devised based on a gradual maturation from the precursor pool of DNA synthesized in the presence of BrUDR. However, a calculation of the quantity of this initial DNA suggests that it is far too small to account for the large number of mutants eventually produced: nor does this hypothesis account for the increasing percentage of mutants with time.

The chemical basis for the mutagenic action of BrU remains uncertain. However, the mutations induced by BrU appear to involve very small molecular changes[22]. One such small change might be the substitution of one base pair for another in the double-helix DNA structure, which would lead to an altered base sequence. (This also has been suggested as a basis for mutation by FREESE[23].) The appearance of spontaneous mutations by the chance occurrence of altered base sequence was originally proposed by WATSON AND CRICK[24] as a corollary to their double-stranded DNA model. Assuming that the incorporation of BrU is a necessary prerequisite for its mutagenic

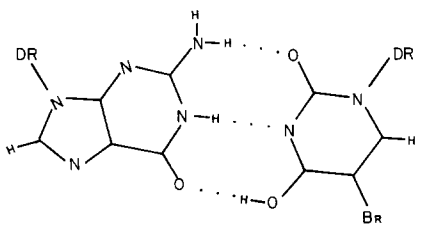

Fig. 6.

action, how might this incorporation lead to changes in the base sequence of phage DNA? In the WATSON AND CRICK DNA structure BrU should, with high probability, pair with adenine to form hydrogen bonds identical to those between thymine and adenine. But BrU might have a small probability of pairing with guanine; though this probability could be great as compared to the probability of thymine pairing with guanine. A hydrogen bonded BrU-guanine pair is pictured (Fig. 6) which assumes that BrU is more stable in the enol form than is thymine (though at the present time there is no evidence to support this assumption). A mutant would be created if BrU was incorporated into DNA opposite guanine and on the next replication adenine was

incorporated opposite BrU. On the other hand, if BrU was first incorporated opposite adenine and on the next replication guanine was incorporated opposite BrU a mutation would also be created. These events would give rise to mutants of different types, since in the former case the final product is the substitution of a guanine-hydroxymethylcystosine pair by an adenine-thymine pair, whereas in the latter case the opposite will be obtained.

The attractiveness of the above scheme is that it is in agreement with the idea that BrU-induced mutations consist of very small chemical changes, and that mutations can appear after free BrUDR is removed. The exchange of one set of base pairs would be sufficient to cause a mutation and the overall chemical composition of a phage lysate containing even a large proportion of mutants need not be detectably altered. It also helps to explain why only about 10 % mutants are obtained even when almost all of the thymines in DNA are replaced by BrU. However, BENZER AND FREESE[22] have reported the striking fact that BrU does not have a random mutagenic effect but induces certain mutations and back mutations[23] much more frequently than others. Therefore, the induction of mutations by BrU cannot be a matter of simple probability of pairing with guanine, but must depend also on the position in the phage genome into which the BrU is incorporated, either because some positions are more susceptible to permanent structure alterations than others or because only a limited number of these alterations cause non-lethal phenotypic effects.

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REFERENCES

1 R. M. LITMAN AND A. B. PARDEE, *Nature*, 178 (1956) 529.
2 R. M. LITMAN AND A. B. PARDEE, *Virology*, 8 (1959) 125.
3 R. M. LITMAN AND A. B. PARDEE, *Biochim. Biophys. Acta*, 42 (1960) 117.
4 R. M. HERRIOTT, in W. D. MCELROY AND B. GLASS, *The Chemical Basis of Heredity*, Baltimore, The Johns Hopkins Press, 1957, p. 399.
5 A. D. HERSHEY, *Advances in Virus Research*, 4 (1957) 25.
6 W. WEIDEL, *Ann. Rev. Microbiol.*, 12 (1958) 27.
7 A. D. HERSHEY AND M. CHASE, *J. Gen. Physiol.*, 36 (1952) 39.
8 E. VOLKIN AND L. ASTRACHAN, *Virology*, 2 (1956) 149.
9 S. S. COHEN, *J. Biol. Chem.*, 174 (1948) 295.
10 S. S. COHEN, *Cold Spring Harbor Symposia Quant. Biol.*, 12 (1947) 35.
11 G. A. VIDAVER AND L. M. KOZLOFF, *J. Biol. Chem.*, 225 (1957) 335.
12 A. D. HERSHEY, *J. Gen. Physiol.*, 37 (1953) 1.
13 A. H. DOERMANN, *J. Gen. Physiol.*, 35 (1952) 645.
14 M. W. BENTZON, O. MAALOE AND G. RASCH, *Acta Pathol. Microbiol. Scand.*, 30 (1951) 243.
15 N. SYMONDS, *Virology*, 3 (1957) 485.
16 S. E. LURIA AND R. LATARJET, *J. Bacteriol.*, 53 (1947) 149.
17 S. BENZER, *J. Bacteriol.*, 63 (1952) 59.
18 A. H. DOERMANN, *J. Bacteriol.*, 55 (1948) 257.
19 S. E. LURIA, *Cold Spring Harbor Symposia Quant. Biol.*, 16 (1951) 463.
20 S. BRENNER AND J. D. SMITH, *Virology*, 8 (1959) 124.
21 L. L. WEED AND S. S. COHEN, *J. Biol. Chem.*, 192 (1951) 693.
22 S. BENZER AND E. FREESE, *Proc. Natl. Acad. Sci. U.S.*, 44 (1958) 112.
23 E. FREESE, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 622.
24 J. D. WATSON AND F. H. C. CRICK, *Cold Spring Harbor Symposia Quant. Biol.*, 18 (1953) 123.