6(*p*-Hydroxyphenylazo)-Uracil: a Reversible, Selective Inhibitor of the Replication of Deoxyribonucleic Acid of Staphylococcal Bacteriophage P11-M15

NEAL C. BROWN

Department of Cell Biology and Pharmacology, University of Maryland School of Medicine,
Baltimore, Maryland 21201

Received for publication 28 July 1971

6(*p*-Hydroxyphenylazo)-uracil (HPUra), a selective inhibitor of the semiconservative replication of deoxyribonucleic acid (DNA) of gram-positive bacteria, was found to inhibit the replication of DNA of bacteriophage P11-M15, a virulent derivative of the temperate *Staphylococcus aureus* bacteriophage P11. At appropriate concentration, HPUra inhibited DNA synthesis by P11-M15-infected *S. aureus* immediately and completely, regardless of the stage of the lytic cycle at which infected cells were exposed to drug. The effect of HPUra was reversible since the capacity of inhibited, infected cells to replicate phage DNA and produce mature phage could be restored by removal of HPUra from incubation media. Concentrations of HPUra which completely inhibited the replication of P11-M15 in its drug-sensitive host did *not* inhibit the replication of this phage or its DNA in several drug-resistant host mutants. HPUra also did *not* inhibit the replication of two other serologically distinct, virulent staphylococcal bacteriophages, P1 and 44AHJD, in drug-sensitive hosts.

6(*p*-Hydroxyphenylazo)-uracil (HPUra) selectively inhibits the division of gram-positive bacteria by inhibiting the semiconservative replication of deoxyribonucleic acid (DNA; reference 2-4).

How HPUra acts is not readily explained on the basis of what is known of the mechanism of bacterial DNA replication. Addition of HPUra to cultures of drug-sensitive organisms in the exponential phase of growth immediately inhibits the progress of the replication point along the parental DNA duplex (2, 4). This inhibitory effect of HPUra is reversible and does not involve a unique action on the process of initiation of replication. (2, 4). HPUra also does not bind to bacterial DNA with high affinity *in vitro* (2). At concentrations which inhibit the semiconservative replication of DNA completely, HPUra does not inhibit the synthesis of deoxyribonucleoside triphosphates, ribonucleic acid (RNA), protein, lipid, or cell wall material (2, 4). HPUra also does not inhibit the action of DNA polymerase *in vitro* (2) and does not apparently inhibit in drug-sensitive bacteria the *non*-semiconservative mode of DNA synthesis involved in dark repair of ultraviolet-irradiated DNA (3).

HPUra can distinguish different types of semiconservative DNA replication possible in drug-sensitive bacteria. For example, in *Bacillus*

subtilis infected with the virulent bacteriophages SP3, SPO2C: (2), and ϕ e (6), HPUra inhibits the residual synthesis of host cell DNA and has no effect on the replication of the DNA of the phage. In this paper, I have used *Staphylococcus aureus* to determine whether HPUra can distinguish bacterial and phage DNA replication in drug-sensitive organisms other than *B. subtilis*. I find that this differential effect of HPUra is dependent on the particular virulent phage used. HPUra does not inhibit the replication of the DNA of bacteriophages P1, or 44AHJD (10), but *does* inhibit the replication of the DNA of bacteriophage P11-M15 (8), a clear plaque mutant of the temperate phage P11. The nature of this inhibition is described below.

MATERIALS AND METHODS

Source and growth of phage and bacteria. Bacteriophage P11-M15, a clear plaque mutant of the temperate phage P11 (8), and its susceptible host, *S. aureus* 8325-4, were the gifts of R. Novick. Bacteriophages P1 (10) and 44AHJD (10) and their respective hosts, *S. aureus* strains P1 and 44AHJD, were provided by E. Rosenblum.

All experiments involving the growth of uninfected and infected *S. aureus* were performed in the 0.3 cy medium of Novick (7) containing one-half the prescribed concentration of β -glycerophosphate. Bac-

terio-phages P11-M15, P1, and 44AHJD were propagated in 0.3 cy medium on their respective hosts and were stored in a gelatin-containing buffer also described by Novick, (7).

Titration of phage and bacteria. Bacteriophage preparations were titered after serial dilution in the minimal salts medium of Spizizen (11). The procedures used were generally those described by Adams (1). A 3-ml amount of 0.3 cy agar (0.5%) containing phage and indicator bacteria were plated as an overlay on 100-mm plates containing 0.3 cy agar (2%). Plaques were scored after development at 37 C for 6 to 15 hr.

Viable cell concentrations were determined by spreading diluted bacterial suspensions directly on to 0.3 cy agar (2%) plates. Absorbance of cultures was measured at 660 nm against a medium blank in quartz cuvettes (10-mm light path) in a Gilford model 240 spectrophotometer.

Selection of HPURA-resistant *S. aureus*. Cells derived from a single colony were harvested in the exponential phase of growth by membrane filtration and suspended at a concentration of 2×10^8 to 5×10^8 cells/ml in melted (45 C) 0.3 cy agar (0.5%). This suspension (3 ml) was hardened as an overlay on 100-mm plates of 0.3 cy agar (2%) containing HPURA at a concentration of 100 μ M. Colonies appearing after 24 to 48 hr of incubation at 37 C were transferred to 0.3 cy agar plates, re-cloned, and examined for HPURA resistance on plates containing HPURA.

Chemicals and reagents. [$2\text{-}^{14}\text{C}$]thymidine, [$U\text{-}^{14}\text{C}$]L-leucine, and [$8\text{-}^{14}\text{C}$]adenine were purchased from New England Nuclear Corp. β -Glycerophosphate, a major component of 0.3 cy medium, was purchased from Calbiochem. HPURA (I.C.I. no. 31584) was generously supplied by B. Langley of Imperial Chemical Industries, Ltd., and purified to chromatographic homogeneity by methods described elsewhere (4); a 20 mM stock solution was prepared in 50 mM NaOH.

Assay of radioisotope incorporation into RNA, DNA, and protein. A membrane-filter fractionation technique, described in detail elsewhere (4), was used; this method employs liquid scintillation counting and is based on a micromodification of the Schmidt-Thannhauser technique described by Roodyn and Mandel (9).

RESULTS

Effect of HPURA on the synthesis of macromolecules in *S. aureus* 8325-4. The action of HPURA on *S. aureus* (Fig. 1) is identical to that observed in *B. subtilis* (2) and *S. fecalis* (4). At a concentration of 100 μ M, HPURA specifically inhibited DNA synthesis. The incorporation of ^{14}C -leucine into hot trichloroacetic acid-insoluble material (part A) was unaffected by HPURA at this concentration, indicating that the synthesis of bulk protein proceeds normally. Incorporation of ^{14}C -adenine into RNA (part B) also was unaffected by 100 μ M HPURA, whereas its incorporation into DNA was completely blocked. Results

FIG. 1. Effect of HPURA on the incorporation of radioactivity into protein, RNA, and DNA of *S. aureus* 8325-4. At time zero, a tracer amount of isotope and HPURA (final concentration, 100 μ M) was added to a culture of bacteria (5×10^7 cells/ml) growing exponentially at 37 C in 0.3 cy agar medium containing supplemental adenine at a concentration of 0.1 mM. (A) Incorporation of ^{14}C -L-leucine (250 $\mu\text{Ci}/\mu\text{mole}$; final concentration; 1.2×10^6 counts per min per ml) into material insoluble in hot trichloroacetic acid. (B) and (C) [$8\text{-}^{14}\text{C}$]adenine (3 $\mu\text{Ci}/\mu\text{mole}$; final concentration; 2.5×10^6 counts per min per ml) was added; incorporation into the alkali digestible (B, RNA) or alkali-indigestible (C, DNA) portion of the cold trichloroacetic acid-insoluble material. Symbols: $\bullet$, control; $\times$, HPURA.

essentially identical to these, but not shown, also were obtained with *S. aureus* strains P1 and 44AHJD.

Inhibition of replication of phage P11-M15. The results depicted in Fig. 2 clearly show that HPURA inhibits the production of phage P11-M15. The absorbance curves (graph B) of cultures infected in the presence of HPURA indicated a concentration-dependent inhibition of cell lysis which was essentially complete at a concentration approaching 50 μ M; at 50 μ M and above, the increase in absorbance of infected cells was essentially identical to that of uninfected cultures depicted in part A.

Inhibition of phage production is also indicated by the burst curves depicted in part C of Fig. 2. In this experiment, *S. aureus* 8325-4 was infected with P11-M15 in the absence and presence of HPURA at a concentration of 100 μ M, rapidly separated from unadsorbed phage, and reincubated in sterile medium containing drug at the original, respective concentration. Cells which were infected, washed, and suspended in the absence of HPURA burst as expected, yielding an average of 100 plaque-forming units per infected center. In contrast, the cells treated similarly in the presence of HPURA failed to produce a detectable burst of phage. However, after dilution of the drug during titration, essentially 100% of the cells present were recovered as infected centers—even after incubation for 1 hr in the presence of HPURA.

Reversibility of HPURA inhibition of phage pro-

FIG. 2. Effects of HPURa on the production of phage P11-M15 by *S. aureus* 8325-4. (A) Effect of HPURa on absorbance of uninfected cultures. At zero time, drug or diluent was added to a culture in the exponential phase of growth (5×10^8 cells/ml), and samples were removed at intervals for determination of absorbance. Numbers on the curves depict the micromolar concentrations of HPURa. (B) Effect of HPURa on absorbance of infected cultures. The conditions were the same as in (A) except that phage P11-M15 was added at a multiplicity of 10 at the time of drug addition. (C) Effect of HPURa on development of phage. After 4 min of incubation with phage P11-M15 (multiplicity of 10) in the absence and presence of HPURa ($100 \mu\text{M}$), cells (5×10^8 /ml) were separated from unadsorbed phage by rapid centrifugation at room temperature and suspended at their original volume in warm (37°C) sterile medium containing the same respective concentrations of HPURa (0 or $100 \mu\text{M}$). Incubation was continued and samples were removed at intervals, rapidly diluted, and titered as described in Materials and Methods. Centrifugation and resuspension of infected cells after the adsorption period required approximately 8 min.

duction. The results of the experiment depicted in Fig. 3 demonstrated that the capacity of drug-treated, infected cells to produce phage could be restored completely by diluting the HPURa to a noninhibitory concentration. In this experiment, cells were infected with P11-M15 at a multiplicity of 10 in the presence of HPURa ($100 \mu\text{M}$), rapidly harvested by centrifugation, and suspended in fresh medium containing drug at the same concentration. At intervals after resuspension, a portion of the inhibited culture was diluted 1,000-fold into warm medium *minus* HPURa, and phage production in the sample was assayed. The burst curves obtained indicated that infected cells could be incubated with an inhibitory concentration of HPURa for periods up to 40 min and still produce normal amounts of phage at a normal rate when drug was diluted to a noninhibitory concentration.

FIG. 3. Reversal of drug inhibition of phage development. Identical cultures of *S. aureus* 8325-4 in the exponential growth phase (5×10^8 cells/ml) were infected in the absence and presence of HPURa ($100 \mu\text{M}$) with phage P11-M15 at a multiplicity of 10 and harvested and suspended as described in Fig. 2C. At zero time (arrow 1), small portions of the control and inhibited cells were diluted 1,000-fold into warm (37°C) sterile medium *minus* HPURa, and phage development was followed as described in Fig. 2C. At 22 and 40 min (arrows 2 and 3, respectively), additional portions of the infected culture incubated in the presence of HPURa were also diluted in medium *minus* drug and assayed for phage development in a similar manner. Solid line: cells infected, harvested, and suspended in the absence of HPURa; broken line: cells infected, harvested, and suspended in the presence of $100 \mu\text{M}$ HPURa.

Relationship of inhibition of phage production to inhibition of phage DNA replication. Figure 4 depicts the effects of HPURa on the incorporation of ^{14}C -thymidine into DNA of uninfected (part A) and P11-M15-infected (part B) *S. aureus* 8325-4. Both populations of cells incorporated considerable amounts of isotope. After 5 min of exposure to phage, the rate of incorporation by the infected control cultures increased considerably above that of the comparable uninfected culture. This increase resulted primarily from the onset of replication of phage DNA, since the bulk (>80%) of the isotope incorporated between 5 and 40 min after infection at this multiplicity could be recovered in the DNA of intact phage after cell lysis (*unpublished results*).

The incorporation of isotope by both cell populations was approximately equisensitive to HPURa; at concentrations of $30 \mu\text{M}$ and above, incorporation was blocked completely. The abil-

FIG. 4. HPura inhibition of DNA synthesis in P11-M15-infected and uninfected *S. aureus* 8325-4. (A) Uninfected cultures. At zero time, ^{14}C -thymidine (8.7×10^6 counts per min per μmole ; final concentration, $12.5 \mu\text{M}$) was added to portions of an exponentially growing culture (5×10^7 cells/ml) together with HPura at the concentrations (micromolar) shown on the graph. Samples were taken for the analysis of radioactivity incorporated into alkali-stable, cold trichloroacetic acid-insoluble material. (B) Infected cells. The experimental conditions were identical to those in (A) except that phage also was added at time zero at a multiplicity of 10.

ity to inhibit incorporation in infected cells indicated that HPura inhibited the synthesis of phage DNA as well as that of the host.

Characteristics of HPura inhibition of phage DNA replication. The results in Fig. 5 indicate that the effect of HPura on the synthesis of phage DNA, like its effect on replication on host DNA (4), is apparently not related specifically to the initiation of replication soon after infection. Addition of HPura at a concentration of $100 \mu\text{M}$ inhibited replication immediately and completely, regardless of the time of addition after infection. If HPura inhibited only an early phase of replication of phage DNA, some residual incorporation of thymidine would be expected after drug addition. The amount of residual synthesis would depend, of course, on the fraction of the cell population supporting active replication of phage DNA at the time of drug addition.

The results of the experiment described earlier in Fig. 3 indicated that the effect of HPura on the development of phage P11-M15 was reversible. The results of the experiment depicted in Fig. 6 indicate that the inhibitory effect of drug on DNA synthesis was also reversible. In this experiment, cells were infected in the presence of HPura ($100 \mu\text{M}$), rapidly separated from unadsorbed phage by centrifugation, and suspended in fresh medium in the presence of drug. At time zero and 24 min after resuspension, samples of the drug-treated cells were rapidly separated from HPura by membrane filtration, suspended in drug-free medium at their original concentration, and examined for their ability to incorporate ^{14}C -thymidine into DNA. The results indicate that cells recovered essentially full capacity to incorporate isotope, even after 20 min of exposure to drug. Analysis of the lysates of these washed cells

FIG. 5. Drug sensitivity of DNA synthesis of P11-M15-infected cultures at different times after infection. At time zero, exponentially growing *S. aureus* 8325-4 was infected in the presence of ^{14}C -thymidine as described in Fig. 4B. At the times indicated by the arrows, HPura was added to portions of the infected culture at a concentration of $100 \mu\text{M}$. Radioactivity was analyzed as described in Fig. 4B. Symbols: ●, incorporation in the absence of HPura; X, incorporation in the presence of HPura.

by isopycnic equilibrium centrifugation in CsCl revealed that more than 85% of the ^{14}C -thymidine incorporated after drug removal was present in DNA of intact phage particles.

Lack of effect of HPura on DNA synthesis and

FIG. 6. Reversal of drug-induced inhibition of DNA synthesis in P11-M15-infected *S. aureus* 8325-4. An exponentially growing culture (5×10^7 cells/ml) was infected at a multiplicity of 10 in the absence and presence of HPURa ($100 \mu\text{M}$). After a 4-min adsorption period, samples of the infected cells were harvested by centrifugation as described in Fig. 2C, and each was suspended in its original volume of sterile warm medium containing the same respective concentration of HPURa. A sample of each of these infected populations was immediately harvested at 37°C by membrane filtration and suspended after washing in the same volume of fresh medium minus HPURa. At time zero, ^{14}C -thymidine (8.7×10^6 counts per min per μmole ; $12.5 \mu\text{M}$) was added to each sample, and incorporation was followed as described in Fig. 4B. At 24 min (arrow), a second sample of the drug-treated cell suspension was freed of HPURa by filtration and assayed in a similar manner. Symbols: $\bullet$, washed control cells; $\times$, washed drug-treated cells.

production of phage P11-M15 in a drug-resistant mutant of *S. aureus* 8325-4. Twenty-two independently derived HPURa-resistant mutants were isolated as described above. All of the mutants grew and supported the production of P11-M15 normally on 0.3 cy agar plates containing HPURa at a concentration of $50 \mu\text{M}$. The parental strain did not divide or support plaque formation under similar conditions.

Figure 7 depicts the effect of HPURa at a concentration of $50 \mu\text{M}$ on DNA synthesis and development of P11-M15 in one of the drug-resistant mutants, 8325-4 (HP-2), in liquid medium. Part A clearly shows that the incorporation of ^{14}C -thymidine was unaffected by this concentration of drug in both infected and uninfected cells; part B shows that HPURa at a concentration of

FIG. 7. Lack of effect of HPURa on DNA synthesis and production of phage P11-M15 in a drug-resistant mutant of *S. aureus* NN450. (A) DNA synthesis. The conditions of infection with phage P11-M15 and the analysis of isotope incorporation into alkali-stable, cold acid-insoluble material were exactly as described for the parental bacterial strain in Fig. 4. ^{14}C -thymidine (10^7 counts per min per μmole) was added at zero time to a concentration of $12 \mu\text{M}$. HPURa was present at a concentration of $50 \mu\text{M}$. Symbols: solid line, uninfected (control) cells; broken line, infected cells; $\bullet$, no drug; $\times$, HPURa. (B) Burst curves in the presence and absence of HPURa. Conditions described in this experiment are identical to those described in Fig. 2 (C) for the parental strain of *S. aureus* NN450. HPURa, where present, was at a concentration of $50 \mu\text{M}$; Symbols: $\bullet$, no drug; $\times$, HPURa.

$50 \mu\text{M}$ also did not affect the production of phage P11-M15 in this mutant.

The failure of HPURa at a concentration of $50 \mu\text{M}$ to inhibit phage production and DNA synthesis by the infected and uninfected mutant was a clear contrast to its inhibitory effects on the parental strain depicted in Fig. 2 (part C) and Fig. 4.

Lack of effect of HPURa on DNA synthesis and phage production by *S. aureus* infected with phages 44AHJD and P1. Bacteriophage P1 of serological group D (10) and phage 44AHJD of serological group G (10) were used to determine whether their replication in suitable host strains was affected by HPURa. Figure 8 summarizes the experiments with phage P1 and its susceptible host, *S. aureus* P1. The results with *S. aureus* 44AHJD and phage 44AHJD were essentially identical and therefore are not shown.

HPURa at a concentration of $50 \mu\text{M}$ inhibited the incorporation of thymidine into DNA of uninfected cells essentially completely, just as it did in uninfected *S. aureus* 8325-4 (cf. Fig. 4, part A). However, the effect of HPURa on isotope incorporation by P1-infected cells clearly differed from that on incorporation by P11-M15-infected cells. HPURa inhibited the initial uptake of isotope, which presumably represents residual replication of host DNA; however, HPURa did not inhibit

FIG. 8. Lack of effect of HPURA on DNA synthesis and production of phage P1. (A) DNA synthesis. A culture of exponentially growing cells (approximately 5×10^7 cells/ml) was infected (zero time) in the presence and absence of HPURA ($50 \mu\text{M}$) at a multiplicity of 10. ^{14}C -thymidine (1.2×10^6 counts per min per μmole) was also added at zero time to a concentration of $12 \mu\text{M}$, and samples were taken for analysis of isotope incorporation as described in Fig. 4. Symbols: solid line, uninfected (control) cells; broken lines, infected cells; $\bullet$, no drug; $\times$, HPURA. (B) Burst curves. Exponentially growing cells (approximately 5×10^7 per ml) were infected with phage P1 at a multiplicity of 10 in the absence and presence of HPURA ($50 \mu\text{M}$). After an adsorption period of 4 min, the infected cells were harvested and incubated exactly as described for P11-M15-infected cells in Fig. 2C.

uptake into phage DNA. The burst curves seen in part B of Fig. 7 also show clearly that HPURA had no apparent effect on the rate of development or the yield of biologically active, virulent progeny.

DISCUSSION

In sensitive gram-positive bacteria, HPURA selectively inhibits the semiconservative synthesis of DNA; the effect of HPURA on the synthesis of macromolecules in *S. aureus* 8325-4 (Fig. 1) clearly depicts this specificity. At least three observations, based on experiments depicted in Fig. 4, 5, and 7, suggest that DNA replication is also the primary site of the inhibitory action of HPURA on the replication of phage P11-M15.

Basically the results depicted in Fig. 4, 5, and 7 show that HPURA inhibits the replication of host DNA and the replication of phage DNA in a very similar fashion. The results depicted in Fig. 4 indicate that the concentration dependence of HPURA inhibition of DNA synthesis is very similar in infected and uninfected cells. Figure 5 shows that HPURA at high concentration inhibits the replication of phage DNA as rapidly and as completely as that of the host. The results of the experiment in Fig. 7 on the replication of P11-M15 and its DNA in the drug-resistant host mutant clearly indicates that mutation in the host

can simultaneously render the replication of both phage and host DNA equiresistant to HPURA.

The action of HPURA on phage DNA replication resembles its action on bacterial DNA replication in at least two respects not mentioned above. First, the effect of HPURA is reversible, as shown by the experiments depicted in Fig. 3 and 6. Second, HPURA apparently inhibits active ongoing synthesis of DNA and does not act by specifically preventing initiation of phage genome replication; this property of HPURA is suggested by the results shown in Fig. 5. HPURA inhibited DNA synthesis of infected cells with the same efficiency both early in the infection cycle and later when considerable DNA replication was in progress. The rate at which HPURA acts (see Fig. 5) is the strongest evidence in favor of a direct effect of HPURA on active replication. This rate was determined more accurately in an experiment employing ^3H -thymidine of high specific activity. The results (not shown) indicated that addition of HPURA at a concentration of $100 \mu\text{M}$ to a culture 20 min after phage infection at high multiplicity inhibited isotope incorporation completely within 10 sec. If HPURA acted solely by preventing the cyclic reinitiation of genome replication, this experiment should have detected the residual incorporation of isotope as actively replicating genomes were completed—even if no more than 5% of the infected cells were supporting phage DNA replication at the time of drug addition.

The physical and functional nature of the HPURA-sensitive process involved in the replication of phage DNA is unknown; functionally, however, it must resemble the process operating in bacterial DNA replication. I have not yet been able to establish firmly whether the phage carries genetic information for its own drug-sensitive function or whether it parasitizes that of the host. P11-M15 is a virulent derivative of the temperate phage P11, and as such may have developed a dependence on the host DNA synthetic process. In fact, two types of somewhat negative evidence suggest such a dependence. First, I have been unable to isolate phage mutants capable of forming plaques on drug-sensitive 8325-4 when the latter is seeded at high density on plates containing HPURA. Second, I have not found among the drug-resistant mutants of *S. aureus* isolated thus far a strain which fails to support phage replication at HPURA concentrations which permit normal bacterial growth.

The failure of HPURA to inhibit the replication of phages P1 and 44AHJD and their DNA clearly indicates that the effects of HPURA depend on the staphylococcal phage used. The basis for this dif-

ference of sensitivity is unclear; apparently, it does not depend on the use of different strains of staphylococci as hosts since each of the strains used was equally sensitive to HPURa. Unfortunately, the rather strict host specificity of these staphylococcal phages prevents a vigorous evaluation of this question by appropriate cross experiments. I have found, however, that phage P1 can replicate with a low efficiency in *S. aureus* 8325-4, the host used for P11-M15; under these conditions, HPURa still had no effects on its replication.

The properties of HPURa described for phage P11-M15 indicate that the drug may be potentially useful in appropriately sensitive systems for characterizing mechanisms of phage DNA replication and the relationship of phage DNA replication to other processes involved in phage formation and maturation. HPURa sensitivity also provides a useful means of classifying phages with respect to a particular type of DNA replication. The usefulness of such a classification will, of course, depend on a knowledge of the structural and functional nature of the drug-sensitive site and the specific mechanism by which HPURa affects it. Studies exploring these parameters are in progress.

ACKNOWLEDGMENTS

I thank Dennis Brown and Lindsay Black for their suggestions and critical reading of this manuscript. It is a pleasure to acknowledge the expert technical assistance of Per-Olof Backman.

This work was supported by Public Health Service research grant CA 11534 from the National Cancer Institute.

LITERATURE CITED

1. Adams, M. H. 1959. Assay of phage by the agar layer method, p. 450-451. *In* Bacteriophages. Interscience Publishers, Inc., New York.
2. Brown, N. 1970. 6(*p*-Hydroxyphenylazo)-uracil: a selective inhibitor of host DNA replication in phage-infected *Bacillus subtilis*. *Proc. Nat. Acad. Sci. U.S.A.* 67:1454-1461.
3. Brown, N. 1971. Inhibition of bacterial DNA replication by 6(*p*-hydroxyphenylazo)-uracil: differential effect on repair and semi-conservative synthesis in *Bacillus subtilis*. *J. Mol. Biol.* 59:1-16.
4. Brown, N., and R. Handschumacher. 1966. Inhibition of the synthesis of deoxyribonucleic acid in bacteria by 6(*p*-hydroxyphenylazo)-2,4-dihydroxypyrimidine I. Metabolic studies in *Streptococcus faecalis*. *J. Biol. Chem.* 241:3083-3089.
5. Maaløe, O., and P. Hanawalt. 1961. Thymidine deficiency and the normal DNA replication cycle. I. *J. Mol. Biol.* 3: 144-155.
6. Marcus, M., and M. Newlon. 1971. Control of DNA synthesis in *Bacillus subtilis* by phage ϕ e. *Virology* 44:83-93.
7. Novick, R. P. 1963. Analysis by transduction of mutations affecting penicillinase formation in *Staphylococcus aureus*. *J. Gen. Microbiol.* 33:121-136.
8. Novick, R. P. 1967. Properties of a cryptic high-frequency transducing phage in *Staphylococcus aureus*. *Virology* 33: 155-166.
9. Roodyn, D., and H. Mandel. 1960. A simple membrane fractionation method for determining the distribution of radioactivity in chemical fractions of *Bacillus cereus*. *Biochim. Biophys. Acta* 41:80-88.
10. Rosenblum, E., and S. Tyrone. 1964. Serology, density, and morphology of staphylococcal phages. *J. Bacteriol.* 88: 1737-1742.
11. Spizizen, J. 1958. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. *Proc. Nat. Acad. Sci. U.S.A.* 44:1072-1078.