[7] In a previous publication,[8] we reported for the older Type II Sigma ribonuclease a value of $\Delta n/c = 0.185$ ml/gm. We now correct this value to 0.187 ml/gm; however, the precision cannot be evaluated with confidence.

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*TWO SEQUENTIAL REPRESSIONS OF DNA SYNTHESIS IN THE ESTABLISHMENT OF LYSOGENY BY PHAGE P22 AND ITS MUTANTS**

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Wild-type phage P22 (hereafter designated c^+) is a temperate phage. On infection of its host, *Salmonella typhimurium*, some cells may lyse, but others survive as lysogenic progeny. Lysogenic bacteria are characterized by the ability to produce the infecting phage, but are immune to subsequent infection by the phage. The controlling element of the lysogenic condition is the prophage, which is the phage genome integrated into the bacterial hereditary material.[1-3]

The c^+ phage produces turbid plaques owing to the growth of lysogenic cells in the plaque center. A larger number of clear-plaque-forming mutants, affected in the ability to become prophage, have been isolated. These fall into three complementation groups: c_1, c_2, and c_3.[4] The class c_3 mutants are still temperate, but give lower frequencies of lysogenization than does c^+. The clear mutants c_1 and c_2 behave like virulent phages, since they lyse all infected cells with liberation of progeny particles. In mixed infection with mutants of the c_2 type, phages of the c_1 class lysogenize as well as the c^+ phage. Genetic studies place these three groups of mutants into three closely linked, but nonoverlapping, complex loci.[5] The complementation exhibited by the mutants of these different cistrons suggests that a number of phage-controlled functions are necessary for the establishment of lysogeny.

This report describes studies of the rate of incorporation of tritiated thymidine into infected cells during the establishment of lysogeny. In experiments with c_1 and c_2 phage, deviations from the pattern of incorporation exhibited by c^+-infected cells allow conclusions as to the functions of the c_1 and c_2 loci. If the rate of H^3-thymidine incorporation is equated to the rate of DNA synthesis, the data may be interpreted as showing that these loci control two sequential repressions of DNA synthesis in infected cells, both repressions being necessary for lysogenization.

Materials and Methods.—Bacteriophage strains: Purified stocks of wild-type (c^+) phage P22 and two complementing clear mutants, c_1^7 and c_2^5, were used in these experiments. A complete description of the characteristics of these mutants and their positions on the phage linkage map has been given.[4, 5]

Bacterial strains: Salmonella typhimurium strain LT2 was used for all infections. A *Gal*– mutant was used as background for plating.

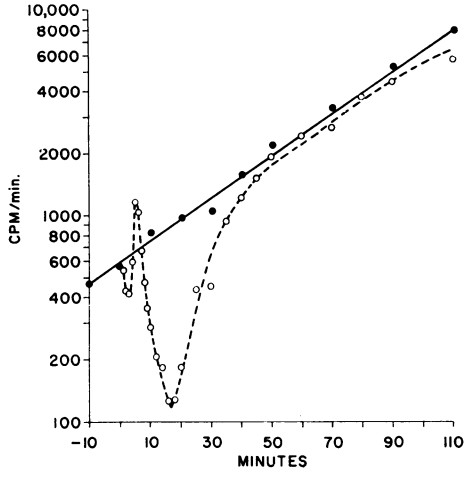

FIG. 1.—The rate of incorporation of H^3-thymidine into phage c^+-infected complexes. ●, uninfected control culture; O, infected cells.

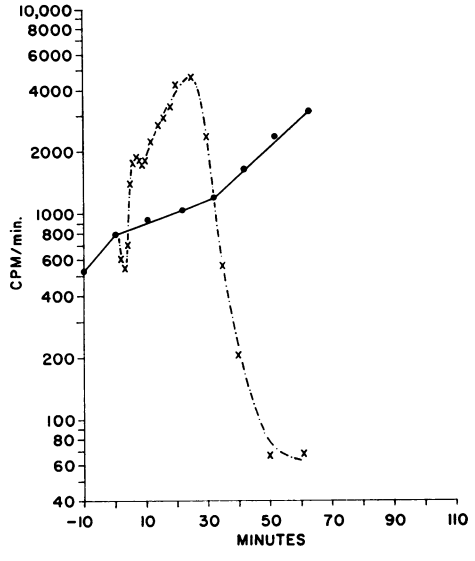

FIG. 2.—The rate of incorporation of H^3-thymidine into phage c_1-infected complexes. ●, uninfected control culture; X, infected cells.

Media: All infections were performed in supplemented M-9 (KH_2PO_4—0.022 *M*, Na_2HPO_4—0.042 *M*, NH_4Cl—0.018 *M*, $MgSO_4$—2.5×10^{-3} *M*, NaCl—8.5×10^{-3} *M*, glucose—0.2% plus 1.5% Difco vitamin-free casamino acids decolorized by filtration through Norit A). Buffered saline, nutrient agar, EMB galactose agar, and soft agar for top layers have been described previously.[4]

Infections: Cells were aerated at 37°C in supplemented M-9 to a titer of approximately $5–10 \times 10^7$/ml. Aliquots were removed at time 0 to tubes containing the appropriate phage to give a multiplicity of infection of about 20 to assure virtually simultaneous infection of all cells.[6] Frequencies of lytic and lysogenic responses were determined by plating on EMB galactose agar.[4] Variations of this basic procedure will be described as they occur.

Determination of the rate of DNA synthesis: The rate of DNA synthesis was estimated by incorporation of H^3-thymidine into acid-insoluble material during a 1-min pulse.[7] Samples of 0.5 ml of bacterial suspension were incubated for 1 min with 0.05 ml of H^3-thymidine (sp. act. = 640 mC/mM; 10.0 μC/ml) at 37°C at specified times. The reaction was stopped by chilling on ice and the addition of 0.2 ml of chilled trichloroacetic acid (17% w/v) containing 2.5 mg/ml unlabeled thymidine. After at least 30 min on ice, the precipitate in each tube was collected on membrane filters (Millipore HA, 0.45 μ pore size) and washed with five 3-ml washes of chilled water. The filters were dried, placed in glass vials containing 15 ml of liquid scintillation medium (toluene—1 liter, PPO—5 gm, POPOP—100 mg), and counted in a Tri-Carb scintillation spectrometer.

Rate of uptake measurements of H^3-thymidine in log-phase cultures were found to be independent of the length of pulse for times up to several minutes. Thus a 1-min pulse was well within the linear range of uptake. Also, for titers below 2×10^8 cells/ml, a direct proportionality between H^3-thymidine uptake and cell concentration was obtained. Variations from proportionality were observed for bacterial concentrations above 2×10^8 cells/ml, so that dilution was necessary in some experiments to maintain growing cultures at approximately 10^8 cells/ml.

Results.—DNA synthesis on infection with phage c^+: A comparison was undertaken of the rates of DNA synthesis in uninfected cells and in phage c^+-infected cells leading to high levels of lysogeny (Fig. 1). The rate of DNA synthesis in uninfected cells increased logarithmically in direct relation with increases in cell titer. In the case of infected cells, uptake followed a different course. At zero time a por-

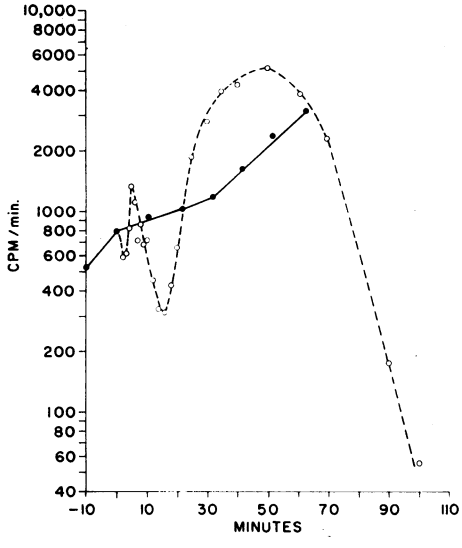

FIG. 3.—The rate of incorporation of H^3-thymidine into phage c_2-infected complexes. These data and those of Fig. 2 are from the same experiment. ●, uninfected control culture; O, infected cells.

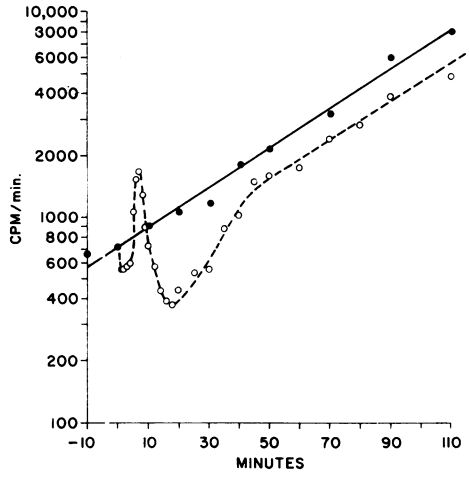

FIG. 4.—The rate of incorporation of H^3-thymidine into cells mixedly infected with phages c_1 and c_2. ●, uninfected control culture; C, infected cells.

tion of the control culture was infected with a multiplicity of 20 c^+ particles which resulted in an immediate depression of H^3-thymidine uptake. This was followed at 3 min by a steep rise in the rate of DNA synthesis, reaching a peak at 5–6 min which was well above the control level of synthesis. At 6 min DNA synthesis fell off rapidly, reaching a low point of only 10–15 per cent of control values at 16–18 min. The rate of synthesis then increased until 40–45 min, when it paralleled that for uninfected cells.

More than 95 per cent of the infected cells survived as lysogenic bacteria. No increase in cell titer was noted for the first 40 min after infection. Thereafter, the titer increased at the same rate as that of control cells, correlating well with the observed resumption of DNA synthesis at normal rates at this time.

DNA synthesis on infection with phage c_1: A striking alteration in the pattern of DNA synthesis was seen on infection with c_1 phage (Fig. 2). After the initial 3-min depression, there is a sustained rise in the rate of DNA synthesis up to 25 min, followed by a rapid fall. A shoulder or minor peak at 6–9 min was seen in most of the phage c_1 curves. This corresponds with the time when c^+-infected cells showed a rapid drop in rate of synthesis. The latent period, marked by the appearance of free phage, was 25 min, correlating well with the peak of DNA synthesis. The final yield of phage was 2.4×10^{10} particles/ml, equivalent to a burst size of 350.

DNA synthesis on infection with phage c_2: A third pattern of DNA synthesis was observed in phage c_2-infected cells (Fig. 3). In its early stages, the c_2 pattern was similar to that of c^+ infections. After the initial depression, a peak of synthesis occurred at about 6 min. This was followed by a sharp decrease in rate of synthesis until 16 min. At this time synthesis resumed, but instead of eventually

paralleling the rate of synthesis of uninfected cells, it rose to severalfold over control levels to a peak at 50 min, after which it fell rapidly. In agreement with delayed synthesis of DNA in the c_2 infection, the latent period was extended. The first phage appeared at 50 min. The final yield of phage was 6.3×10^{10} particles/ml, giving a burst size of approximately 1000.

DNA synthesis on mixed infection with phages c_1 and c_2: Mixed infection with 10 particles each of c_1 and c_2 phages gave over 90 per cent lysogeny. In agreement with expectation, the pattern of DNA synthesis (Fig. 4) in these mixedly infected cells was typical of that of c^+-infected cells.

The nature of DNA synthesized during the early minutes of phage c^+ infection: The rate of H^3-thymidine incorporation in the early peak (at 6 min, Fig. 1) reached levels above that of control cells, suggesting that predominantly phage-specific DNA synthesis occurred at this time. Further support for this contention derives from experiments now to be described.

The rationale of these experiments is as follows. If the early peak of synthesis is indeed phage-controlled, infections carried out in bacteria in which host DNA synthesis is inhibited should also exhibit a peak at 6 min. Phage-specific synthesis should not be affected by the inhibition of host DNA synthesis. On the other hand, a change in the peak should be seen on infecting cells with phages which cannot replicate normally. These conditions are met by using starved, ultraviolet-inactivated cells in the first instance and ultraviolet-inactivated phage in the second.

Logarithmically growing strain LT2 bacteria at 2×10^8 cells/ml were washed twice and aerated for 1 hr in buffered saline. A sample of the starved cells was exposed for 1 min to a 15-watt General Electric germicidal lamp at a distance of 50 cm, a dose which gave less than 0.1 per cent survival. After 10 min for adsorption of c^+ phage in buffered saline, cells were centrifuged and resuspended in M-9 at 5×10^7 infected complexes/ml. Uninfected irradiated cells showed no DNA synthesis, the uptake of label being effectively at background levels (Fig. 5, curve *C*). In contrast, phage infection of these cells (Fig. 5, curve *D*) resulted in a distinct peak of DNA synthesis at 6 min, strongly supporting the association of this peak with phage DNA synthesis.

Phage c^+, suspended in buffered saline, was irradiated for 7 min at a distance of 50 cm, a dose giving a survival of 10^{-4}. Log-phase cells were infected with a multiplicity of 20 original particles and the rates of DNA synthesis determined (Fig. 6). In cultures infected with ultraviolet-irradiated phage, there was a substantial reduction in the early peak, again supporting the phage nature of this synthesis.

Two other aspects of the experiments involving ultraviolet treatment should be commented upon. (1) Infections of irradiated cells with normal phage invariably led to the lytic response rather than lysogeny. The rapid rise in the rate of DNA synthesis in curve *D*, Figure 5, was a reflection of the lytic response. (2) The heavily irradiated phage killed host cells without releasing any appreciable quantity of phage—in agreement with the absence of recovery of labeled-thymidine uptake in these cells.

Discussion.—Earlier studies on DNA metabolism of temperate phage-infected complexes utilized chemical methods which measure the total amount of DNA present at any one time. With the use of such methods it was shown that, on infection of *E. coli* K12 with phage λ, very little DNA was synthesized for the first

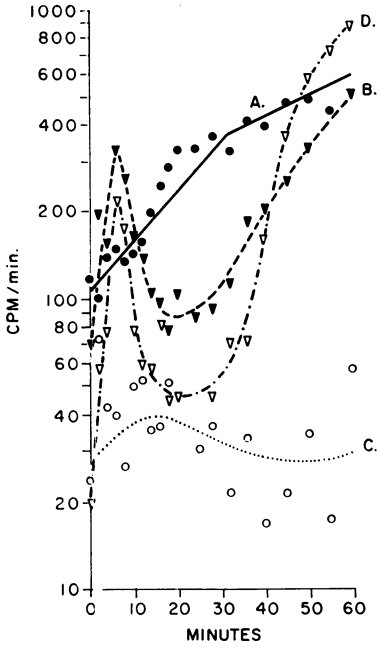

FIG. 5.—The rate of incorporation of H^3-thymidine into ultraviolet-irradiated cells infected with c^+ phage. Curve *A*, unirradiated uninfected control cells. Curve *B*, unirradiated infected cells. Curve *C*, irradiated uninfected control cells. Curve *D*, irradiated infected cells.

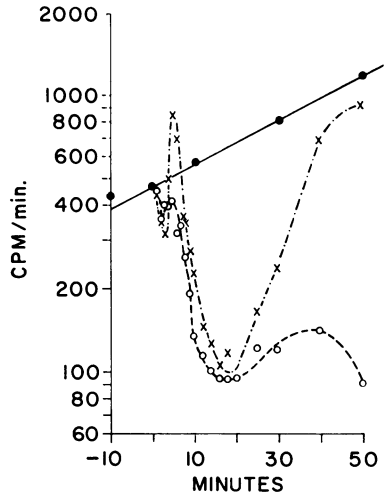

FIG. 6.—The rate of incorporation of H^3-thymidine into cells infected with ultraviolet-irradiated phage c^+. ●, uninfected control cells; X, cells infected with untreated phage; O, cells infected with ultraviolet-treated phage.

30 min, but then synthesis resumed in parallel with cell replication.[8] Similar results were obtained with phage P22.[6] These techniques, however, are not sensitive to subtle changes in DNA synthesis over relatively short intervals. Pulse labeling is admirably suited to detection of such shifts in that it estimates the rate of synthesis at any given time, in effect ignoring the DNA already present. It should be clear, however, that the rate of incorporation of label into acid-insoluble material is an accurate measure of the true rate of DNA synthesis only when entry of labeled thymidine into the cell and equilibration with the endogenous pool are not rate-limiting. While it cannot be positively stated that these conditions obtain here, the excellent correlation of physiological events such as cell growth, phage production, and cell lysis with the rate curves supports this.

All infections of log-phase cells show an immediate depression in the rate of DNA synthesis. The significance of this is not understood, nor will it be commented upon further, since it is not essential for the subsequent discussion. At about 3 min, a sharp rise in the rate of synthesis in c^+-infected cells occurs and reaches a peak at about 6 min. Data from the UV experiments, Figures 5 and 6, strongly suggest that this early peak of DNA synthesis is largely phage-specific in origin. This burst of synthesis is short-lived. There follows a marked depression in incorporation of radioactive thymidine which persists until the sixteenth minute, when the rate of synthesis rises sharply again. Once inhibition sets in at 6 min, autonomous phage replication seems to remain inhibited in cells giving lysogenic progeny. Clearly, host DNA synthesis is also inhibited, as the over-all rate of tritiated thymidine

incorporation continues to drop, approaching zero rate at about 16 min. DNA synthesis begins again, reaching rates similar to those of uninfected control cells. This is undoubtedly a release of host DNA synthesis from inhibition as the cells begin to divide normally, and phage DNA replication presumably continues to be inhibited thereafter.

Two sequential functions are required for the inhibition of autonomous phage replication in the establishment of lysogeny. Each function is under the control of an independent phage locus. This conclusion derives from the specific patterns of the rates of DNA synthesis in cells infected with mutants c_1 or c_2. The ability to inhibit phage DNA synthesis at 6 min is controlled by the c_1 locus. This early inhibition is not in itself sufficient for lysogenization. A second function, under the control of the c_2 locus, is required to maintain the inhibition beyond 16 min, when cellular synthesis is released. Failure of either of these functions results in vegetative phage replication. In the case of c_1 mutants, the rate of phage DNA synthesis continues to rise past 6 min, going on to lysis and liberation of progeny particles at 25 min. On the other hand, c_2 mutants show the 6-min inhibition, followed by massive synthesis of phage DNA starting at 16 min and lasting until the end of the latent period at 50 min. It is interesting to note that the lytic responses of these two mutants show such different patterns of synthesis.

A comparison of the areas under the early (6-min) peak and the later (50-min) peak as described in Figure 3 for c_2-infected cells suggests that something in the order of 20–30 phage equivalents are synthesized in the first burst of DNA synthesis. This represents approximately a single replication for each injected genome. Luria *et al.*[9] also found evidence for limited replication of phage genomes in P22 infections leading to lysogeny. Some replication of phage DNA may be requisite for integration of prophage.

Clear-plaque-forming mutants have been reported for other temperate phages.[10–13] Genetic and functional phenomena remarkably similar to that described for phage P22 appear to be operating in coliphage λ. Three different phenotypes of clear mutants which fall into three closely linked genetic loci have been recognized. The mutants of each locus complement those of the others. The c_1 locus of phage λ (analogous to the c_2 of P22) determines the immunity specificity of the phage[14] and also controls the production of a repressor of phage production.[15] The analogy between the c regions of P22 and λ is strengthened by the demonstration that the c_2 region of P22 also controls phage replication. Further, complementation between the phage λ clear-plaque mutants occurs even when infection with a c_2 type (analogous to the c_1 of P22) follows infection with c_1 (analogous to the c_2 of P22) by 20 min, but not in the reverse order.[10] These findings are interpretable in terms of the sequential action of the P22 clear mutants on DNA synthesis. The λc_1 mutant carries out the early inhibition normally but fails in the late one. The λc_2 phage can provide the late inhibition, and if it does so before 20 min, lysogeny can be established. When infection takes place in the reverse order, the c_2 type does not carry out the early inhibition, committing the cells to lysis at an early stage. Thus, the similarities between the P22 and λ systems are many. The most obvious inconsistency at the moment is that the c region of phage P22 does not appear to control the immunity specificity. Zinder[16] has evidence for an immunity locus near one end of the phage P22 linkage map.

The sequential actions of the c_1 and c_2 loci are, in effect, repressions of autonomous phage replication in cells producing lysogenic progeny. These phenomena can be visualized in two ways. (1) The c_1 locus initiates the repression, and the c_2 locus maintains it; both loci remain active in preventing vegetative phage formation. (2) The c_1 locus initiates the repression, but acts only for a limited time. An independent repression is then initiated by the c_2 locus. This would imply that the two loci truly act in sequence, the first function no longer being needed to maintain lysogeny once the second is active. However, the continued activity of the second function is required for perpetuation of the lysogenic condition from then on.

The evidence supports the second of these hypotheses. As has been demonstrated, both repressions occur when a cell is mixedly infected with c_1 and c_2 mutants and high levels of lysogeny are achieved. The prophages of these lysogenic cells are very stable and are always of the c_1 type, never the c_2.[4] Clearly, the normal functioning of the c_1 locus is not needed to maintain lysogeny once an early stage is passed. The failure to find stable c_2 prophages suggests strongly that prolonged action of this locus is necessary for continued maintenance of lysogeny. Studies involving temperature-sensitive c_1 and c_2 mutants substantiate these conjectures and will be reported elsewhere.[17]

The mechanisms by which these repressions are accomplished are not understood. The c_I locus of phage λ controls the production of a cytoplasmic repressor of vegetative phage synthesis.[15, 18] The complementation seen in mixed infections with phage P22 c mutants suggests that the observed repressions are also mediated through cytoplasmic substances since the wild-type alleles are in *trans* position in these complexes.[18] Whether these "repressors" act at the genic or enzymatic level remains to be determined. In this connection, it would be of interest to examine the *in vitro* DNA synthesizing capability of extracts of cells showing the extreme repression at 16 min after c^+ infection.

The genetic control of the repression and subsequent release of host DNA synthesis remains in question. It is conceivable that the c_1 and c_2 loci are pleiotropic in that they not only control repression of phage replication but also, respectively, control repression of host DNA synthesis and its eventual release. That other phage loci are involved in control of host synthesis would seem a more likely possibility. A search for such loci is in progress.

Summary.—The rate of DNA synthesis in complexes infected with wild type and mutants of phage P22 was followed by H^3-thymidine pulse labeling. The essential features of the c^+ infection, which gives high-frequency lysogeny, are: (1) early onset of phage-specific DNA synthesis, continuing until the sixth minute of the infection; (2) a sharp depression in the over-all rate of DNA synthesis, both phage and host, starting at the sixth minute, reaching nearly zero rate at about the sixteenth minute; (3) recovery of host synthesis which eventually parallels the rate of uninfected control cells at about 45 min, when infected cells begin to divide and give rise to lysogenic progeny. Cells infected with c_1 mutants, which give only a lytic response, fail to show the depression of synthesis at 6 min. The rate continues to increase rapidly until about 25 min when the cells begin to lyse and liberate progeny particles. On the other hand, c_2-infected cells, which also give only lysis, do show the early inhibition, which is followed by a sharp rise at 16 min which greatly exceeds control rates, reaching a peak at 50 min when cell lysis starts. Mixedly in-

fected cells, which produce high frequency of lysogeny, show a pattern of DNA synthesis similar to that of c^+-infected complexes. The functions of the c_1 and c_2 loci are interpreted as controlling DNA synthesis during infection leading to lysogeny. The c_1 locus represses phage DNA synthesis in the first few minutes of the infection, and the c_2 locus maintains the repressed state after the recovery of cellular replication.

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† Postdoctoral research fellow of the U.S. Public Health Service.

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INTEGRATED MAGNETIC AND SUPERCONDUCTIVE MEMORIES

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Communicated by V. K. Zworykin and read before the Academy, April 29, 1964

1. *Introduction.—Memory in computers:* Electronic techniques allow the execution of millions and even billions of arithmetic or other elementary logic functions in minutes or at worst in hours. This great speed is significant only for long sequences of computations, and sequences that are strictly specified by detailed instructions. These instructions must be available at speeds compatible with those of the computation, and it must be possible to select automatically among alternative instructions according to the results of computation in progress.

The intermediate numbers occurring in a long numerical computation and the instructions forming the program for the execution of the problem must be stored. This is the role of the "memory." It must be possible to dispose of incoming information, or to "write-in" the "memory," at any desired location, or "address,"