chlorinated hydrocarbon insecticides in an assay method with mosquito larvae (*6*). They found no instances in which the joint action could be classified as either antagonistic or synergistic. Aldrin, which is chemically related to dieldrin, was reported to act independently of DDT. Turner, however, showed that dieldrin plus DDT resulted in increased toxicity toward adult milkweed bugs and found evidence of "interaction" (*7*). The pairing of other compounds was also reported to give enhanced toxicity. The results reported in this paper might appear to contradict the studies of toxicity in insects because the amount of dieldrin stored was reduced, not enhanced, by the presence of DDT. The reduced accumulation of dieldrin in the tissues, however, might mean that greater quantities were circulating and available for specific toxic action. In that case, the different reports might be reconcilable. It remains to be determined whether the toxic effects of dieldrin are enhanced by the presence of DDT in mammals, as was reported by Turner (*7*) for an insect species.

Alternatively, the effect of DDT on dieldrin storage may be related to the rat's ability to transform dieldrin into readily excreted hydrophilic products (*8*). Such a mechanism would be of potential significance in dealing with hazards arising from the toxicity of dieldrin.

Clearly, data obtained by studying single compounds in mammals and pairs of compounds in insects may not be indicative of the behavior of mixtures of compounds in mammals or their pharmacological effects.

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Sequential Gene Action in the Establishment of Lysogeny

Abstract. *A study of temperature sensitive clear-plaque-forming mutants of bacteriophage P22 demonstrates that the* c_1 *and* c_2 *loci must function in a temporal sequence in the establishment of lysogeny in* Salmonella typhimurium. *The* c_1 *locus acts for only a 4-minute interval between the 7th to 11th minute of the infection. The* c_2 *locus begins to function some minutes later, and its continued activity is necessary for perpetuation of the lysogenic state.*

The establishment of the lysogenic condition on infection of *Salmonella typhimurium* strain LT2 with bacteriophage P22 requires two sequential repressions of phage DNA synthesis. These conclusions derive from studies on the rates of DNA synthesis as measured by incorporation of H^3-thymidine into acid-insoluble material during 1-minute pulses in complexes of bacterial cells infected with the wild-type, c^+ phage and its clear-plaque-forming mutants, c_1 and c_2 (*1*). Under appropriate conditions, infections with c^+ phage result in almost 100 percent lysogenic responses; in contrast, infections with either c_1 or c_2 produce 100 percent lytic responses. These mutants complement to give high frequencies of lysogeny (*2*). At 37°C, phage-associated DNA synthesis (*1*) begins rather early in c^+ infected cells and continues until the 6th minute of the infection, when a sharp repression in the overall rate of synthesis sets in. This early repression persists until the 16th minute, at which time host-specific DNA synthesis is released from inhibition. The rate of synthesis then increases until it reaches the same rate of increase and parallels the rate of synthesis in uninfected control cells at about 45 minutes. At this time the infected cells begin to divide and produce lysogenic progeny.

Striking alterations in this pattern of DNA synthesis are seen on infection with either of the two clear-plaque-forming mutants. Cells infected with c_1 mutants do not exhibit the repression of synthesis at 6 minutes. The rate continues to increase until about 25 minutes have elapsed, when the cells start to lyse and liberate phage. Cells infected with mutant c_2 do exhibit the early inhibition, but a sharp rise in the rate of DNA synthesis occurs at 16 minutes which greatly exceeds control rates; the synthesis reaches a peak at 50 minutes, when cell lysis begins. These mutants complement one another in mixed infection, and the pattern of DNA synthesis is similar to that of c^+ infected complexes. The functions of the c_1 and c_2 loci are to control phage DNA synthesis in the establishment of lysogeny. The c_1 locus represses phage DNA synthesis at the 6th minute of the infection, and the c_2 locus maintains the repressed state from the 16th minute onward as cellular replication resumes. We now report studies with temperature-sensitive c_1 and c_2 mutants which confirm and extend the conclusions reached from the studies on the rates of DNA synthesis. By shifting infected cells between low (31°C) and high (42°C) temperature it is possible to pinpoint the time of onset and duration of action of these loci in the establishment of lysogeny.

A lysate of phage c^+, produced in the presence of 100 γ of bromodeoxyuridine per milliliter of M-9 medium supplemented with casamino acid (*1*), was used as the source for selection of temperature-sensitive, clear-plaque-forming mutants. These mutants were detected by plating large amounts of treated phage on nutrient agar and incubating at 42°C. Phage from clear plaques was isolated and tested by plating for each mutant at both 31° and 42°C. Most of these isolates formed clear plaques at both temperatures and

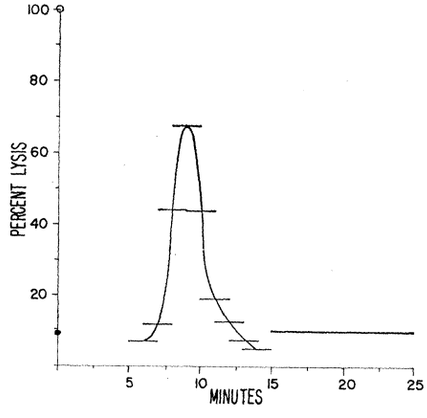

Fig. 1. The effect of 2-minute heat pulses at various times after the onset of the infection on the lysogenic responses of cells infected with temperature-sensitive c_1 mutants of bacteriophage P 22. ●, Frequency of lysis by control infections at 31°C; ○, frequency of lysis by control infections at 42°C; short dash, frequencies of lysis after a heat-pulse at indicated intervals; long dash, frequency of lysis after a high-temperature treatment from the 15th to the the 25th minute.

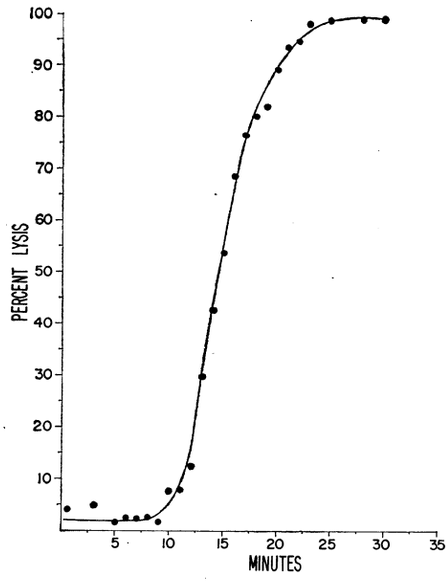

Fig. 2. The effect of exposure to 42°C on the lysogenic responses of cells infected with temperature-sensitive c_2 mutants. At the indicated times after the beginning of the infection, samples were transferred from 42°C to 31°C and plated to determine the frequency of lysis and lysogeny.

were not temperature-sensitive mutants. Approximately 5 percent gave wild-type turbid plaques at 31°C, but clear plaques at 42°C, this being the phenotype which distinguishes temperature-sensitive mutants. Several dozen of these mutants were readily isolated by this procedure and further characterized.

Sensitive cells, each infected in liquid medium with about 20 particles of these mutants, gave at least 80 percent lysogenic responses at 31°C. The same infections carried out at 42°C produced 100 percent lytic responses. All mutants could be classified as either the c_1 or c_2 type by spot testing for complementation (*2*) at 42°C against standard c_1 and c_2 mutants. Once classified as to c type, these showed patterns of rates of incorporation of tritiated thymidine at 42°C typical of the class. Lastly, the mutants map in the appropriate c regions as judged from preliminary recombination studies. A representative c_1 and c_2 temperature-sensitive mutant was chosen for further study.

After equilibration at 31°C, a log-phase culture was infected with 20 particles of the temperature-sensitive c_1 mutant per bacterial cell. After an adsorption period, the treated complexes were diluted and distributed into 0.2 ml portions in small test tubes. Beginning at 5 minutes, samples were placed in a 42°C water bath for 2-minute treatments at 1-minute intervals during the course of the infection. After the 2-minute exposure to high temperature, the samples were replaced in the 31°C water bath, plated on eosin methylene blue (EMB) galactose agar plates and incubated at 31°C. Plating on this medium permits a determination of the number of infected cells which give the lysogenic or lytic response (*2*).

Heat treatments during the first 7 minutes of the infection produced no change in the frequency of the lysogenic response of temperature-sensitive c_1 infected complexes. Cells treated at any time up to the 7th minute (either by continuous or 2-minute treatments) showed the same frequency of lysogeny, 90 percent (only 10 percent lysed), as did control cells maintained at all times at 31°C (Fig. 1). Two-minute heat pulses overlapping the interval of 7 to 11 minutes resulted in significant shifts to lysis; the greatest effect of all, approximately 70 percent lysis and only 30 percent lysogeny, was produced by the heat pulse from 8 to 10 minutes. No amount of heat treatment after 11 minutes affected lysogenization. In addition, prolonged culture at 42°C of lysogenic cells carrying this temperature-sensitive c_1 prophage failed to cause a shift to the lytic response. Once established as prophage these are as stable as are wild-type prophages; they are not inducible by high temperature. Similar results were obtained using 3-minute heat pulse treatments, the peak treatment reaching 100 percent lysis.

Bacteria in the log phase of growth were infected with 20 particles of the temperature-sensitive c_2 mutant per cell at 42°C. At 1-minute intervals, platings were made on EMB galactose agar, and the plates were incubated at 31°C to determine the frequency of lysis and lysogeny. Incubation at 42°C for the first 11 minutes of the infection did not affect the response of the cells infected with the temperature-sensitive c_2 mutant (Fig. 2). All exposures to high temperature, including continuous exposure for the entire 11 minutes, showed less than 10 percent lysis and better than 90 percent lysogenization. An increase in the frequency of lysis and a concomitant decrease in lysogeny was observed on exposure of cells to 42°C for more than 12 minutes. Each additional minute at 42°C caused a greater shift to lysis until 100 percent lysis was achieved at about 25 minutes. Application of high temperature at any time thereafter caused induction of phage production in these cells.

The time of onset of temperature sensitivity in infections with these mutants corresponds very well with the time of onset of the repressions of phage DNA synthesis already described. For the c_1 locus, repression starts at about 6 minutes in infected complexes maintained at 37°C; temperature sensitivity appears at about the 7th minute at 31°C. For the c_2 locus, at 37°C, repression starts at about the 16th minute, while temperature sensitivity begins at about the 12th minute in infected complexes maintained at 42°C. The disparities in timing are unquestionably due to the differences in the temperatures of the cultures. Both approaches strongly support the conclusion that the two loci start to function at different times: the c_1 locus early and c_2 locus later.

These studies provide additional information concerning the duration of action of these loci. The c_1 locus not only starts to act early but needs only function for a short but critical interval, 3 to 4 minutes, in order to accomplish its part in the establishment of lysogeny. After the 11th minute of the latent period, the activity of the c_1 locus is not needed to maintain the lysogenic state as demonstrated by the inability of high temperature to induce phage production in cells lysogenic for temperature-sensitive c_1 mutants. In contrast, once it is activated, the c_2 locus must continue to function for perpetuation of the lysogenic state. Cells lysogenic for temperature-sensitive c_2 mutants are induced by appropriate treatments with high temperature. The activities of these loci are, then, sequential. The early c_1 repression is analogous to the paraimmunity function postulated by Zinder (*3*). The P22 temperature-sensitive c_2 mutant appears to be of the same class as the inducible temperature-sensitive mutants of bacteriophage λ (*4*) and the c_2 locus is undoubtedly responsible for the immunity characteristic of the lysogenic cell.

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