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*EFFECT OF ULTRAVIOLET IRRADIATION OF BACTERIOPHAGE T2 ON ENZYME SYNTHESIS IN HOST CELLS**

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The infection of *Escherichia coli* by bacteriophage T2 results in the appearance of a number of novel enzyme activities which cannot be detected in the host cell prior to this event.[1–5] In addition, infection is followed by an increase in level of certain constituent enzyme activities of the bacterium.[2, 6, 7]

Studies of phage-induced enzyme production as a function of the time after infection have shown that after a lag period of 2 to 5 min the levels of these enzymes increase linearly up to 10 to 12 min after infection and thereafter tend to remain at a more or less constant value.

Flaks *et al.*[8] have shown that the concentration of deoxycytidylate hydroxymethylase measured 15 min after infection of *E. coli* with ultraviolet irradiated phage T2 remains essentially unaltered within certain doses of radiation while phage production decreases logarithmically under these conditions.

In this laboratory, a more detailed study has been made of the production of this enzyme as a function both of time after infection and of dose of ultraviolet light on the infecting phage. It has been shown that up to a certain level of radiation there is indeed little or no effect detectable in the kinetics of deoxycytidylate hydroxymethylase production up to 10 or 12 min after infection. Beyond this time, however, a rather striking divergence between the behavior of the irradiated samples and that of the unirradiated control becomes apparent. Instead of the normal cessation of enzyme formation, the samples infected with phage treated with ultraviolet light exhibit a continued linear increase up to 30 to 60 min after infection. The results of these experiments are presented below.

Methods and Materials.—Irradiation of phage T2: During irradiation phage T2Hr$^+$ were suspended in glucose-ammonium chloride medium[9] at a concentration of 4.7 to 5.5 $\times$ 10^{11} particles per ml. Three ml of the above phage suspension in quartz cuvettes were irradiated for the intervals indicated with stirring. The unfiltered output of a 4-watt General Electric germicidal lamp was used as the source of radiation. The survival of the phage was determined from plaque counts obtained by plating suitable dilutions of T2 suspension before and after irradiation. The plates were incubated in the dark to prevent photoreactivation.

Infection of E. coli with irradiated phage T2: *E. coli* B was grown in the glycerol-

casamino acid medium of Fraser and Jerrel[10] with vigorous aeration to a concentration of approximately 1×10^9 cells per ml. The culture was chilled to prevent further growth. Aliquots of 10 ml of bacterial culture were placed in chilled 500 ml Erlenmeyer flasks. Prior to the addition of phage each flask was placed on a shaker in a water bath at 37°C and preincubated for one min. Then 0.1 ml of phage T2 suspension was added (approximately 5×10^{11} infectious phage per ml before irradiation). Aliquots of 1 ml were removed from each incubation vessel at the times indicated and immediately frozen by pipetting them into liquid nitrogen. The frozen aliquots were stored at −20°C for 1 to 2 days. To release the enzymes the cells were disrupted in a Hughes press.[11] The extracts obtained in this manner were used without further purification for enzyme assays.

Determination of efficiency of adsorption of phage by E. coli: Just prior to the preincubation period an aliquot of the chilled *E. coli* culture was plated for colony count. Five min after infection 0.1 ml aliquots were removed and rapidly diluted into chilled nutrient broth, an aliquot of which was quickly removed and spread on agar for colony counts of viable *E. coli*. In the samples treated with either unirradiated or irradiated phage 3 to 4 per cent of the original *E. coli* input could be recovered as viable cells after adsorption of phage had been allowed to take place.

The maximum number of infected bacteria that liberate phage after infection with phage irradiated for one min was measured. It was found to be less than 1.5 per cent of that of a culture infected with untreated phage.

Enzyme assays: Deoxycytidylate hydroxymethylase was assayed by measurement of the incorporation of C^{14}-labeled formaldehyde into dHMP.[‡] The reaction mixture, similar to that described by Flaks and Cohen,[12] contained in 0.5 ml total volume: tris (hydroxymethyl) aminomethane buffer (acetate salt), pH 8.0, 25 μmoles; ethylenediamine tetraacetate, pH 8.0, 10 μmoles; dCMP, 2.1 μmoles; 2-mercaptoethanol, 10 μmoles; tetrahydrofolate,[13] 0.45 μmole; C^{14}-labeled formaldehyde, 1 μmole with a specific activity of 0.62 μcurie per μmole; enzyme extract containing the material from 4×10^7 cells. After incubation at 37° for 45 min, the reaction was terminated by chilling the vessels and adding 1 ml of a solution which was 0.2 *M* with respect to unlabeled formaldehyde and 1.85×10^{-5} *M* with respect to carrier dHMP. The enzyme was then inactivated by heating on a boiling water bath for 2 min. The reaction mixture was added to a Dowex 1-8x formate ion exchange column (3.3 mm in diameter × 50 mm in length; 200–400 mesh resin). After adsorption of the solution, the column was washed with 5 ml of water followed by 8 ml of 0.015 *M* ammonium formate buffer, pH 3.0. Elution of dHMP was accomplished with an additional 8 ml of the same buffer. The entire eluate containing the dHMP was dried on an aluminum planchet and the radioactivity was measured.

The assay for dCTPase has been previously described.[14] The assay for dTMP kinase was performed by incubation of enzyme and C^{14}-labeled dTMP under the conditions described by Lehman *et al.*[15] followed by separation of the radioactive dTTP by means of a Dowex-1-formate ion exchange column.

Results and Discussion.—Figure 1 represents a plot of the values of deoxycytidylate hydroxymethylase levels versus time after infection of *E. coli* with phage T2 irradiated with varying doses of ultraviolet light. Inspection of this graph reveals (*a*) that with the lower doses of ultraviolet light (curves B and C) there is very

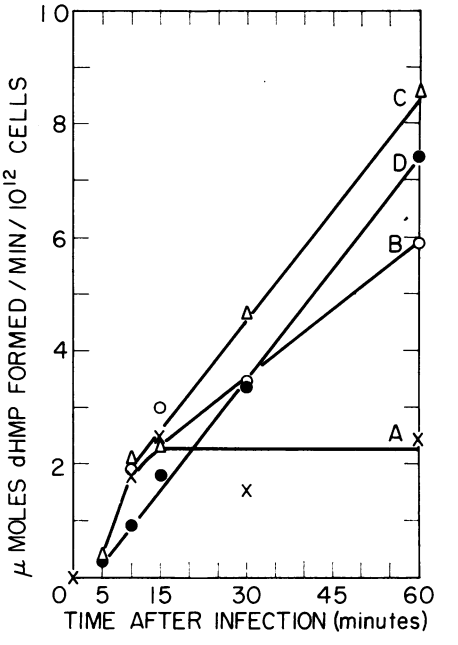

FIG. 1.—Deoxycytidylate hydroxymethylase activity as a function of time after infection of *E. coli* B with phage T2 irradiated with varying doses of ultraviolet light.
E. coli input, 8×10^8 cells per ml.
Phage input, 4.7×10^9 particles per ml.

Designation on graph	Time of irradiation (min)	Fraction of plaque forming phage after irradiation
X	0	1
O	0.5	1.2×10^{-3}
△	1	7.2×10^{-6}
●	1.5	2.8×10^{-7}

little, if any, change in the time of appearance of the enzyme and in the initial rate of its production as compared to that in cells infected with unirradiated phage (curve A); (*b*) that with all cells infected with irradiated phage a linear increase in enzyme concentration is observed after 15 min of infection, whereas in cells treated with unirradiated phage no change of enzyme level occurs after this time; (*c*) that the portion of the curves (A, B, and C) representing the enzyme levels beyond the first twelve min after infection show an increase in slope as the radiation dose to the phage is increased; (*d*) that at higher doses of ultraviolet light (curve D) the initial rate of production of the enzyme is slower than that in the control cells infected with unirradiated phage (curve A); however, the final value of enzyme activity in the cells infected with irradiated phage exceeds that of the control since no decline in the rate of enzyme production after 15 min occurs in the former case.

In Figure 2 are presented data of an experiment in which the hydroxymethylase synthesis by cells infected with unirradiated phage is compared with that by cells infected with phage irradiated for 1 min. The synthesis was followed over the duration of 4 hr. The results of this experiment confirm those shown in Figure 1. Again in the case of cells infected with unirradiated phage the level of enzyme reached a maximum at approximately 10 min and then remained constant for the duration of the experiment. In contrast, the cells infected with irradiated phage continued to produce enzyme at the initial rate for 30 min, after which time there was further enzyme formation but at a slower rate.

The most striking feature of these data comparing the effects of irradiated and unirradiated phage is the divergence of the pattern of enzyme synthesis beyond 10 min after infection. These results are consistent with the hypothesis that ultraviolet irradiation of phage causes a lesion in some system responsible for cessation of enzyme formation during normal infection.

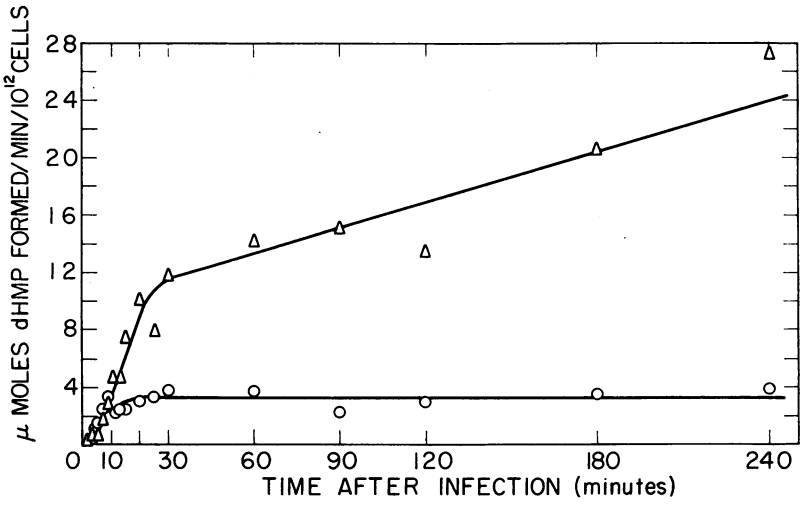

FIG. 2.—Deoxycytidylate hydroxymethylase activity as a function of time after infection of *E. coli* B with unirradiated and irradiated phage T2.

E. coli input, 1×10^9 cells/ml.
Phage input, 4.6×10^9 particles/ml.
Time of irradiation: 1 min.
Concentration of phage during irradiation: 4.6×10^{11} particles/ml.
Fraction of phage surviving irradiation: 8×10^{-6}.

The volumes of *E. coli* culture, phage suspension and incubation flask were 4 times those given under *Methods and Materials*.

○ unirradiated phage T2
△ irradiated phage T2

In the period before 10 min the rate of enzyme production is affected only when the infecting phage were exposed to the highest dose of ultraviolet light; under such circumstances the initial rate of enzyme formation is lower than that which occurs after infection with untreated phage. These facts are presented in favor of the argument that the system responsible for the initiation of enzyme synthesis, although radiation sensitive, is more resistant than the system responsible for arrest of the synthesis during the later period.

Preliminary investigations into the possible generality of this phenomenon for other phage-induced enzymes have been carried out by assaying for dCTPase,[2, 3] another enzyme detectable only after phage infection, and for dTMP kinase,[2, 6] an enzyme which is present in uninfected cells but which greatly increases after infection. In both these cases enzyme formation continues beyond the initial 10- to 12-min period whenever infection is produced by irradiated phage, as in the case of hydroxymethylase. With these two enzymes, however, the initial rate of enzyme formation is significantly decreased at a radiation level (1 min) which does not effect the initial rate of hydroxymethylase formation. Thus, it appears that the factor or factors responsible for the initial formation of dCTPase and of dTMP kinase are more sensitive to ultraviolet light than that for the induction of hydroxymethylase. These differences are illustrated in the case of dTMP kinase by the data shown in Figure 3.

The cause for prolonged enzyme synthesis in the host cells upon infection with

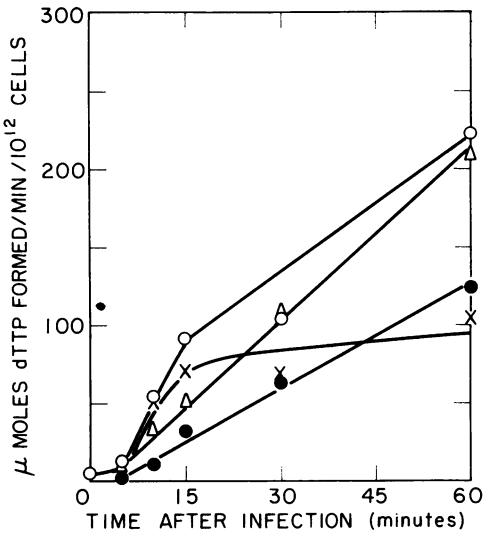

FIG. 3.—Thymidylate kinase activity as a function of time after infection of *E. coli* B with phage T2 irradiated at varying levels with ultraviolet light. The assays were performed on the same extracts as those for deoxycytidylate hydroxymethylase presented in Fig. 1.

Designation on graph	Time of irradiation of phage (min)
X	0
○	0.5
△	1
●	1.5

irradiated phage has not been determined. In searching for an explanation for this phenomenon two major questions may be posed. (1) Does infection of *E. coli* by unirradiated T2 cause the formation in the host cell of a chemical entity which has the function of controlling the levels of induced enzymes directly? Or (2) does the appearance or accumulation in sufficient quantity of any one of the components required for phage formation play an indirect regulatory role in enzyme synthesis? Among substances which might be involved in such an indirect role, DNA, phage structural proteins, or phage lysozyme may be mentioned. New phage DNA first appears 7 min after normal infection[16, 17] but fails to do so when infection is caused by irradiated phage.[8, 17] Structural proteins[18, 19] and lysozyme can be detected at about 10 min after infection. Such an indirect control could be exerted by the physical presence of these entities. Alternatively, the initiation of the synthesis of the structural proteins and lysozyme during infection by the unirradiated phage may result in some manner in the diversion of metabolic precursors from the synthesis of the early enzymes to that of these other proteins.

The fact that infection with irradiated phage results in a greater level of these enzymes in comparison to infection with unirradiated phage is of practical value in their isolation.

Summary.—The kinetics of formation of deoxycytidylate hydroxymethylase, an enzyme induced in *E. coli* by infection with phage T2, have been studied. Under the usual conditions of infection, enzyme formation takes place during the time interval of 3 to 12 min after infection, after which time the enzyme level remains approximately constant. If the cells are infected with phage which have been irradiated with ultraviolet light at an appropriate dose, the kinetics of enzyme formation during the first ten minutes are similar to those observed upon infection with unirradiated phage. However, enzyme formation continues for a longer period of time after infection with the irradiated phage. This effect has also been noted with two other phage-induced enzymes, deoxycytidine triphosphatase and thymidylate kinase. Possible implications of this finding are discussed.

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† Postdoctoral fellow of the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service (1959–1960).

‡ The following abbreviations are used: dCMP, dCTP, the mono- and triphosphate, respectively, of deoxycytidine; dTMP, dTTP, the mono- and triphosphate, respectively, of thymidine; dHMP, deoxy-5-hydroxymethylcytidylate; dCTPase, deoxycytidine triphosphatase; DNA, deoxyribonucleic acid.

[1] Flaks, J. G., and S. S. Cohen, *Biochim. Biophys. Acta*, **25**, 667–668 (1957).

[2] Kornberg, A., S. B. Zimmerman, S. R. Kornberg and J. Josse, these PROCEEDINGS, **45**, 772–785 (1959).

[3] Koerner, J. F., M. S. Smith, and J. M. Buchanan, *J. Am. Chem. Soc.*, **81**, 2594–2595 (1959).

[4] Somerville, R., K. Ebisuzaki, and G. R. Greenberg, these PROCEEDINGS, **45**, 1240–1245 (1959).

[5] Keck, K., H. R. Mahler, and D. Fraser, *Arch. Biochem. and Biophys.*, **86**, 85–88 (1960).

[6] Bessman, M. J., *J. Biol. Chem.*, **234**, 2735–2740 (1959).

[7] Flaks, J. G. and S. S. Cohen, *J. Biol. Chem.*, **234**, 2981–2986 (1959).

[8] Flaks, J. G., J. Lichtenstein, and S. S. Cohen, *J. Biol. Chem.*, **234**, 1507–1511 (1959).

[9] Adams, M. H., in *Bacteriophages* (New York: Interscience Publishers, Inc., 1959) p. 446.

[10] Fraser, D., and E. A. Jerrel, *J. Biol. Chem.*, **205**, 291–295 (1953).

[11] Hughes, D. E., *Brit. J. Exptl. Pathol.*, **32**, 97–109 (1951).

[12] Flaks, J. G., and S. S. Cohen, *J. Biol. Chem.*, **234**, 1501–1506 (1959).

[13] Blakley, R. L., *Biochem. J.*, **65**, 331–342 (1957).

[14] Koerner, J. F., M. S. Smith, and J. M. Buchanan, *J. Biol. Chem.*, (in press).

[15] Lehman, I. R., M. J. Bessman, E. S. Simms, and A. Kornberg, *J. Biol. Chem.*, **233**, 163–170 (1958).

[16] Hershey, A. D., J. Dixon, and M. Chase, *J. Gen. Physiol.*, **36**, 777–789 (1953).

[17] Vidaver, G. A., and L. M. Kozloff, *J. Biol. Chem.*, **225**, 335–347 (1957).

[18] De Mars, R. I., *Virology*, **1**, 83–99 (1955).

[19] Koch, G., and A. D. Hershey, *J. Mol. Biol.*, **1**, 260–276 (1959).

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*A POSSIBLE ROLE FOR ARGININE IN ENZYME MECHANISMS**

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It has been proposed that a histidine residue plays an essential part in the catalytic activities of a number of enzymes.[1] The evidence cited in support of this hypothesis is extremely suggestive but, on the whole, indirect. A major part can be summarized as follows:

(*a*) The pH-activity curves of a large number of enzymes suggest the participation of a group having a pK_a in the region of 6.5. The imidazolium ion dissociates in this region; the ionizable functional groups of other amino acids normally do not.

(*b*) Certain monoacylated enzymes, such as acetyl chymotrypsin, are labile, losing their acyl group rapidly in neutral and slightly basic solutions. Acylated imidazole compounds behave in a similar manner.

(*c*) Imidazole derivatives are capable of catalyzing the hydrolysis of nitrophenyl esters and, when suitably designed, will catalyze the hydrolysis of other esters and amides.