BACTERIOPHAGE T_4 INDUCED MODIFICATION OF VALYL-tRNA SYNTHETASE IN *ESCHERICHIA COLI*. AN ANALYSIS OF THE KINETICS AND REGULATION.

by

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TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

One of the important goals in cellular physiology is the understanding of processes that lead from the recognition of genetic information to the construction of a fully active biomolecule. Several steps in this process are known and comprehensively described as transcription and translation. Their regulation, however, is only partially understood. One approach to understanding the mechanisms of regulation of protein synthesis is to study the characteristics of aminoacyl-tRNA synthetases (36). These enzymes are indispensible elements in the pathways leading from an array of 20 amino acids to highly complex protein configurations. Because of their unique interaction with nucleic acids on the one hand and amino acids on the other, they occupy a key position in protein synthesis.

At least one of the cellular functions of aminoacyl-tRNA synthetases is the generation of charged transfer RNA. The cognate amino acid is first converted to the aminoacyl adenylate by a pyrophosphorolytic split of ATP and then attached to the respective tRNA with the release of AMP. Mg^{2+} ions are required for both the activation and the transfer step (36). Because of their indispensible function, the characteristics of these enzymes are somewhat unique and their regulation is thought to be different than the regulation of other biosynthetic enzymes (37).

Aminoacyl-tRNA synthetases are present in high concentrations in bacterial cells. This seems to be somewhat independent of the cells demand for these molecules. Approximately 4% of all cellular proteins and 10% of the cell's enzymatically active protein is represented by these enzymes (37, 58). They are involved in the control of stable RNA synthesis and repression and derepression of amino acid biosynthetic enzymes (16). Rather

unique is the 1:1 molar ratio of tRNA : aminoacyl-tRNA synthetase in the bacterial cell. These properties of amino acid activating enzymes suggest that their regulation must be different and more sophisticated than those control mechanisms known for biosynthetic pathway enzymes.

Although early evidence suggested that synthetases were present in constant amounts and that their synthesis was constitutive (58), Nass demonstrated in 1967 that at least phenylalanyl-tRNA synthetase and isoleucyl-tRNA synthetase were subject to specific repression and derepression when the endogeneous supply of phenylalanine or valine respectively was manipulated (34). Since the rate of synthetase formation for the histidine and leucine enzyme remained uneffected under similar conditions, a fundamental difference in the regulation of different aminoacyl-tRNA synthetases seemed possible.

Using a density labeling technique to distinguish preexisting from newly formed enzyme, Williams demonstrated two years later that arginyl- and histidyl-tRNA synthetase were regulated by a repression-like mechanism (58). From these findings it was concluded that probably all synthetases are subject to regulation on the level of transcription. The fact that these repression-derepression mechanisms could be detected for synthetases only when special conditions were employed was explained by an extensive _in vivo_ inactivation of these enzymes in the chemostat experiments of Nass (58). This situation became more clear by the work of Anderson _et al._ (1972), who proposed a mechanism by which the valyl-tRNA synthetase (VRS) of the mutant NP-2907 undergoes inactivation as a consequence of its functioning. The rate of VRS inactivation was shown to be dependent upon the ratio of deacylated to acylated tRNA, which is a function of the growth rate (2). A different aspect of the regulation of amino acid activating enzymes was observed by

Parker in 1972 by determining the specific activity of arginyl, leucyl, and valyl-tRNA synthetase as a function of the growth rate. Upon a shift from minimal to casamino acid supplemented media, he observed a 2.5 fold increase in specific activity, which he termed metabolic regulation (40).

In 1966 Neidhardt et al. utilized a set of aminoacyl-tRNA synthetase mutants of E. coli to see whether the bacteriophage T_4 depends upon host synthetases for the synthesis of viral proteins (38). After infection with this phage they noted the appearance of a new VRS activity in the mutant NP-29 (formerly called I-9) which possessed a thermolabile valine enzyme. This new enzymatic activity appeared to be heat stable and enabled the mutant to charge tRNA and synthesize protein at higher non-permissive temperatures. Similar phage effects could not be demonstrated for the glycine, histidine and phenylalanine mutants. Instead they found e.g. an absolute requirement of the phage for the host phenyl-tRNA synthetase (12).

A more detailed study of the phage induced VRS activity was carried out by Neidhardt et al. in 1969 (39). A comparison of the host and phage related enzymes revealed that the phage enzyme had a greater resistance to denaturation by heat or urea, a larger molecular volume, a higher sedimentation rate in sucrose gradients, a greater net positive charge and a greater ability to charge valine to yeast tRNA. Most of the other synthetases were examined after phage infection with regard to these properties, but except for the valine enzyme no alteration in the physico-chemical properties of the host enzymes could be detected with the available techniques. These studies did not include the synthetase activities for asparagine, cysteine, glutamine and proline.

Several observations suggested that the phage induced synthetase activity resembled a modification of the host enzyme rather than a de novo

synthesis of a phage specific synthetase. Neidhardt and Earhardt had shown that protein synthesis was absolutely required for the phage effect (38). With a density shift technique, however, it was found that the "phage enzyme" consisted mainly of polypeptide chains made prior to infection (39). The idea of a conversion mechanism was strongly supported by the finding of Crispeels et al. in 1968 that the loss of host activity and appearance of phage enzyme were coordinated when crude extracts of cells at different stages of infection were subjected to sucrose density gradient centrifugation (10). These studies suggested a time course of approximately 5 minutes for the appearance and 15 to 20 minutes for the total conversion of host to modified enzyme. It was further indicated that modification of host VRS might play a possible role in the differential regulation of "early" versus "late" protein synthesis in phage infected cells.

A bacteriophage T_4 mutant, designated T_4rI364 vs2, was then isolated that was unable to induce the conversion of the host enzyme to a urea resistant form (39), and was further characterized by McClain et al. (31). He showed that the conversion of host enzyme to a T_4 specific form was controlled by a phage gene, termed (vs), located between the rI and e loci on the linkage map of T_4. The product of this gene appeared to be a peptide, since the vs mutation could be suppressed by growth on an E. coli strain containing an amber suppressor. Subsequently this peptide was found to associate with the host VRS during modification (31, 39). The fact that T_4 vs mutants could grow on E. coli wildtype strains with the same fidelity as wild type phages suggested that the ability to convert the host valyl-tRNA synthetase to a urea resistant form is a nonessential function under laboratory conditions.

Further characterization of the vs gene product was carried out by Marchin et al. (29, 30) who used a double labeling technique to introduce carbon 14 and tritium into host and phage specific proteins respectively. Under these conditions 10% of the modified VRS molecule was synthesized after infection with the phage. Chromatography of the modified enzyme under denaturating conditions resolved the molecule into two fragments, the host enzyme and the T_4 specific component, which was termed τ-factor (30). A comparison of wildtype and modified VRS revealed a molecular weight for the modified form of 120,000, when chromatographed on G-200 Sephadex (29). Enzymological differences such as the value of the K_m for ATP, valine or tRNA could not be detected.

Characterization of the phage specific factor τ revealed that this molecule had protein-like properties with a molecular weight of approximately 10,000. After combining with the host VRS it conferred unique physical properties upon this enzyme. Denaturation of the VRS-τ complex and reattachment of τ to native VRS could be achieved, but did not rule out the possibility that other components were necessary for normal modification.

Several questions remain unsolved, such as the regulation of (vs) gene expression, the kinetics of appearance of modified VRS and the physiological significance of modification. The object of this thesis is to report experimental data that bear strongly on the first two questions.

Bacteriophage T_4 is a relatively complex DNA phage with a double stranded circular permutated and terminally redundant chromosome. With a molecular weight of 1.3×10^8 daltons this genome is large enough to code for nearly 200 genes, of which approximately 80 have been defined mostly by conditional lethal mutants (32). The work of Stahl and his colleagues

revealed the existence of a large number of polar groups or transcription units on the linkage map of T_4 (48, 49), whose differential expression appear to be regulated by unknown mechanisms. The phage life cycle is initiated by a two step attachment of the phage to a rather specific site on the E. coli cell wall (50). After adsorption to the cell wall the phage becomes irreversibly fixed. That fixation is temperature sensitive and rate limiting at high bacterial concentrations (17). By a rather sophisticated mechanism involving a confirmational change of the sheath, tail plate and tail fibers the core penetrates the cell wall and injects the DNA content of the head (32). This process alters the permeability of the cell wall, resulting in an extensive leakage of cell contents during the first few minutes of infection. Using T_1 and T_2 phage at a multiplicity of infection (MOI) between 5 and 30, Puck and Lee found that some RNA and even the enzyme β-galactosidase pass through the cell wall (41, 42). This leakage is halted by a sealing reaction, which is induced by the phage 3 to 5 minutes after entering the host (32). Although Silver et al. showed that the ability of the cell to take up potassium or magnesium ions from the medium is not affected by the phage (47), the report of Guttman et al. of a T_4 induced magnesium pump in infected cells (21) suggests a change of the magnesium ion concentration within the cell.

Shortly after the viral DNA penetrated the cell wall, host specific m-RNA and protein synthesis are arrested (9) and the E. coli DNA is degraded (25). After a period of 4 to 7 minutes phage specific DNA synthesis is initiated (7, 11) and reaches its maximum rate about 10 minutes after infection.

Characteristic for the nucleic acid synthesis of T_{even} phages is the incorporation of 5-hydroxymethylcytosine into the DNA, and a modification of

the completed strands by methylation and glucosylation (32). Since 5-hydroxymethylcytosine as well as most of the modifying enzymes are not present in the uninfected cell, the phage genome must carry the information for the synthesis and regulation of these essential enzymatic activities. The complex pathway of DNA synthesis, which involves at least 20 different phage specific enzyme activities (32) and the finding that essentially no structural components are synthesized before DNA synthesis is initiated indicates the existence of temporal regulatory mechanisms responsible for the controlled expression of phage genes in infected cells.

These control systems were comprehensively studied by Hosoda and Levinthal in 1968, who used a method combining electrophoresis and autoradiography to study protein synthesis in infected E. coli cells (22). By growing and infecting cells at 37°C they were able to define two classes of phage specific proteins: those whose synthesis starts before 7 minutes after infection were designated as "early" proteins, and those which were produced after that time were called "late" proteins. For a more detailed analysis of these classes infection was carried out at a temperature of 25°C. Under these conditions four groups of proteins could be defined: class A proteins, made from 0 to 10 minutes, class B proteins, made from 0 to 20 minutes, class C proteins, made between 5 and 25 minutes and class D, synthesized from 20 to 45 minutes. While all "late" proteins started to be synthesized at the same time (class D), the "early" ones appeared to be more individualistically regulated and fell into at least three classes (A, B and C). Furthermore Hosoda and Levinthal demonstrated that DNA synthesis was required but not sufficient for "late" protein synthesis, and that the products of gene 33 and 55 were also required for class D expression.

Similar studies on the timing of T_4 gene expression were done by Salser, Bolle and Epstein, who determined different steps in phage development by the differential appearance of distinct classes of m-RNA (45). By using RNA-DNA hybridization competition techniques with RNA samples removed at different times after phage infection they were able to show that at least 3 different classes of phage specific m-RNA (A, B and C) were synthesized at 30°C during the first 5 minutes of infection. When protein synthesis was blocked with chloramphenicol during this time of transcription, only class A m-RNA was produced in normal amounts, while the synthesis of class C and part of class B RNA was inhibited. Because of their "turn off" in the late period these three classes of messenger were termed "true early." Another class of m-RNA which was present in small amounts at 5 minutes but increased 6 fold late in infection was called "quasi late." "True late" m-RNA's were shown not to appear in the early period (0.04%), but increased 1000 fold in the period of 5 to 20 minutes after infection.

Later on the designation for the "true early" class of m-RNA was altered by calling those messengers transcribed in the absence of concomitant protein synthesis "immediate early" and those that required protein synthesis for transcription "delayed early" (32, 45). The logical conclusion to be drawn from these results was that translation of one or more "immediate early" genes was required for the onset of delayed early gene transcription.

Several laboratories have investigated the events that led from "immediate early" to "delayed early" gene transcription. The work of Bautz *et al.* (3) has revealed that the *E. coli* RNA polymerase without sigma factor transcribes T_4 DNA without any specificity *in vitro*. Upon addition of sigma factor, however, only "early" m-RNA is transcribed. With the finding of

Richardson that E. coli termination factor rho prevents the transcription of T_4 delayed early genes (43), a possible control mechanism for the switch from immediate to delayed early genes could be proposed. An immediate early gene product could act as antitermination factor, which antagonizes the action of rho, thereby allowing the host polymerase to proceed directly from the immediate early to the delayed early region (9, 45). Yet a different mechanism seemed to be possible, supported by several observations: Zillig et al. found that the RNA polymerase subunits became modified after T_4 phage infection (46); Audrey Stevens reported an association of T_4 specific peptides with the core polymerase (51); Grasso et al. found in vivo evidence for the existence of a rapidly induced phage protein for the initiation of class II RNA synthesis (18), and finally Travers isolated a sigma-like T_4 specific factor (54). Thus the translation of an immediate early transcript could modify or associate with the core enzyme to allow the turn-on of delayed early genes. The presence of both a T_4 specific sigma and a T_4 induced antitermination factor is not mutually exclusive.

Concomitant with these studies on the level of transcription several laboratories investigated the regulatory mechanisms in T_{even} phages by monitoring the pattern of early enzyme induction. Shortly after Flaks and Cohen published the work on the induction of deoxycitidylate hydroxymethylase in bacteriophage T_6 infected cells (14), several other enzyme activities were found to be increased or newly synthesized after phage infection (5, 24, 32).

Wiberg et al. studied the regulation of early enzymes in 7 T_4 amber mutants and reported that the failure to induce phage specific DNA synthesis was due to the lack of two early enzyme activities (57). Similar mutant studies by Warner and Lewis (56) indicated the existence of different control

mechanisms for the phage specific enzymes dihydrofolic acid reductase and deoxycytidylate deaminase. Important work for the understanding of these mechanisms came out of the laboratory of Buchanan, who used specific inhibitors of RNA and protein synthesis to divide the infectious cycle in a period of transcription and translation. With this system he demonstrated the presence of control systems regulating the extent and rate of transcription and translation processes in early enzyme synthesis (20), the involvement of DNA replication in cessation of early enzyme expression and transcription of late regions (27), and finally the existence of three early enzyme classes in bacteriophage T_4 infected E. coli cells (26). However, in vitro studies of the kinetic order of early enzyme synthesis revealed that a classification of early proteins based on the appearance of their messenger in the presence or absence of different inhibitors of protein synthesis is still somewhat incomplete and might only partially reflect the complexity of the control mechanisms involved (35, 55).

The basis for my work then resides on these premises. Aminoacyl tRNA synthetases are important regulatory enzymes in the bacterial cell. The preservation of a gene for modification of valyl-tRNA synthetase in E. coli therefore must bear importantly on the survival of T_4 bacteriophage. It was our hope to examine the regulation of the modification reaction and study it in the context of the appearance of other T_4 phage induced enzymatic activities.

MATERIALS AND METHODS

Bacteria and bacteriophage. Escherichia coli NP4 a B strain, NP2 a K strain and NP29 a temperature sensitive mutant derived from NP2, were obtained from Dr. F. C. Neidhardt at the University of Michigan. NP29 grows normally at 30°C but fails to grow at 40°C due to thermal inactivation of its valyl-tRNA synthetase (VRS) (6, 13). Bacteriophage T_4Bc^+ which has no cofactor requirement for adsorption was used exclusively in all experiments.

Media. Cells were grown in a variety of media specifically indicated for each experiment. M9-minimal media containing 5.8 mg/ml Na_2HPO_4, 3 mg/ml KH_2PO_4, 0.5 mg/ml NaCl, 1 mg/ml NH_4Cl, 10^{-5} M $FeCl_3$, 10^{-3} M $MgSO_4$ and 0.4% glucose as carbon source was prepared according to Adams (1). Casamino acid media contained 0.05 M Na_2HPO_4, 0.1 M KH_2PO_4, 10^{-4} M $CaCl_2$, 10^{-3} M $MgSO_4$, 0.015 M $(NH_4)_2SO_4$, 1% casamino acids. Benzer Broth containing 7 g NaCl and 13 g tryptone per 1000 ml H_2O. Glycerol-casamino acid medium was prepared following the procedure of Fraser and Jerrel (15), and contained 10.5 g Na_2HPO_4, 4.5 g KH_2PO_4, 3 ml 0.1 M $CaCl_2$, 1 g NH_4Cl, 0.3 g $MgSO_4$, 15 g casamino acids, 100 ml of a 30% glycerol solution and 1 ml of a 1% gelatine solution in 1000 ml H_2O.

Chemicals. All common chemicals were reagent grade. Adenosine 5'-Triphosphate (ATP), Glutathione (GSH), Bovine Serum Albumin (BSA), 2'-Deoxycytidine 5'-Monophosphoric Acid (dCMP), Triphosphopyridine Nucleotide Reduced (NADPH), 5-Methyl DL-Tryptophan, Chloramphenicol, Lysozyme, and Rifampicin were purchased from Sigma Chemical Company, St. Louis, Missouri. Soluble Ribonucleic Acid (tRNA) extracted from E. coli strain B was purchased from General Biochemicals, Chagrin Falls, Ohio, and utilized

without any further purification. Thymidine 5'-Monophosphate Ammonium (Methyl-^{3}H), spec. activity 39 C/mMole, 50% ethanol, and L-Valine (2,3-^{3}H), spec. activity 6 C/mMole, 0.01 N HCl soln. were purchased from Schwarz/Mann, Orangeburg, New York. AG-I-X8 ion exchange resin (200-400 mesh) was purchased from Bio Rad Laboratories, Richmond, California. Disodium Ethylenediaminetetraacetate (EDTA) was obtained from Fisher Scientific Company, Fair Lawn, New Jersey, and Bacto-Tryptone from Difco Laboratories, Detroit, Michigan. Standard buffer contained 6 mM Potassium-Phosphate and 6 mM β-Mercaptoethanol, pH 7.2.

Preparation of cultures and crude extracts. 100-200 ml cultures were grown aerobically on a Gyrotory Water Bath Shaker and their optical density measured in 5 ml cuvettes in a Klett Summerson Photoelectric Colorimeter using a red filter. At a density of 6 x 10^{8} cells/ml the cultures were infected with bacteriophage T_4Bc^{+} at a multiplicity of infection (MOI) of 3-5 phages per cell. Before phage addition as well as different times after infection 10 ml samples were withdrawn and pipetted into centrifuge tubes precooled to -15°C in a salt-ice solution or containing 1 ml of a chloramphenicol solution (1100 µg/ml) at 0°C. After centrifugation for 10 minutes at 10,000 rpm the pellets were resuspended in 2 ml standard buffer. Cells were sonicated 3 times for 10 seconds in 10 second intervals with a Branson Sonifier (output 60 watts). During sonication the samples were cooled in ice. Cell debris was removed by centrifugation at 10,000 rpm for 10 minutes, and the supernatant referred to as crude extract. The extracts were stored at 4°C and the protein content determined by the method of Lowry et al. (28).

Sucrose gradient density centrifugation. Linear gradients of 5 to 20% sucrose in standard buffer were prepared, mixing 2.3 ml of each sucrose solution in a two-chambered mixing device. 0.2 ml of 5% sucrose plus 0.2 ml of crude extract were layered on top of each gradient and centrifugation was carried out in a Beckman L2-65 ultracentrifuge at 40,000 rpm for 16 hours using the swinging bucket rotor SW 50.1. Approximately 80 fractions were collected through the bottom of the tube. VRS activity was measured by adding 0.45 ml reaction mix directly to every second fraction. Urea stability was analyzed by incubation of every other fraction with 0.05 ml of 8 M urea before subsequent addition of 0.40 ml of reaction mix. Both incubations were carried out at 37°C for 5 minutes.

Assay for VRS activity and urea stability. The urea stability test includes the determination of VRS activity before and after treatment with 4 M urea, as described by Marchin et al. (29). 0.4 ml of a 1:4 dilution of crude extract was added to a preheated mixture (37°C) containing 0.1 ml BSA (1 mg/ml), 0.2 ml 10 M urea and 0.1 ml standard buffer. After 5 minutes incubation 0.1 ml of the incubation mixture was added to the reaction mixture at 0°C, containing 0.5 mg of tRNA, 0.1 mg BSA, 1.0 µmole of GHS, 1.0 µmole of ATP, 10 nmoles of ^{3}H-valine at 15 µCi/µmole, 5 µmoles of KCl, 50 µmoles of Tris-HCl, pH 7.2 and 5 µmoles $MgCl_2$ in a total volume of 0.4 ml.

0.1 ml of a 1:20 dilution of crude extract was added to a different tube containing 0.4 ml of reaction mixture at 0°C. Both reaction mixtures containing urea treated or untreated extract, respectively, were incubated in a waterbath at 37°C for 5 minutes, chilled quickly to 0°C and mixed with 3 ml of 5% Trichloroacidic acid (TCA). After 30 minutes the precipitates were

collected on fiberglass filters and counted in a Beckman LS 100 liquid scintillation counter.

A comparison of the resulting counts per minute of the urea treated and the corresponding untreated enzyme allowed the expression of enzyme activity after treatment with 4 M urea in %, which was termed urea stability. Since modified (urea stable) enzyme appeared to be 50% as active as unmodified (urea unstable) enzyme, all values were corrected for this difference in specific activity.

Assay for dihydrofolic acid reductase. The activity for this enzyme was assayed by the procedure of Warner and Lewis (56). The reaction mixture contained 60 µmoles phosphate buffer, pH 6.9, 24 µmoles β-mercaptoethanol, 0.225 µmoles dihydrofolic acid, and 0.18 µmoles NADPH in a total volume of 2 ml. 200 µg of protein were added at room temperature and the change in absorbance measured in a Gilford Spectrophotometer Model 222, at 340 nm. A reaction mixture containing everything but dihydrofolic acid was used as blank. The reaction was monitored for 5 minutes and the reaction velocity expressed as the slope of the resulting curve. Specific activity was expressed as slope per mg protein.

Assay for deoxycytidylate deaminase. Enzyme activity was determined as described in Warner and Lewis (56). 3 µmole $MgCl_2$, 4 µmole β-mercaptoethanol, 20 µmole Tris-HCl buffer, pH 8.1, 0.4 µmole dCMP, 0.1 µmole dCTP, 1 µmole NaF, and about 200 µg protein in a total volume of 0.5 ml were incubated at 37°C. Aliquots of 0.7 ml were removed after 1, 30, and 60 minutes and mixed with 2.5 ml of 0.1 N HCl. The change in absorbance at 280 nm and 260 nm was measured in a Gilford spectrophotometer and the ratio $A_{280}:A_{260}$ plotted

versus the time. The slope of this curve per mg protein was used to express the specific activity.

Assay for deoxynucleotide kinase. Deoxynucleotide kinase activity was measured using ^{3}H-dTMP as substrate, as described by Lembach et al. (26). The reaction mix contained 20 µmoles Tris-acetate, pH 7.5, 2.0 µmoles $MgCl_2$, 1.0 µmole ATP, 0.15 µmole ^{3}H-dTMP, specific activity 3 x 10^5 cpm/µmole, and 50 µg extract in a total volume of 0.3 ml. The blank contained everything but extract.

After 10 minutes incubation at 37°C the tubes containing the reaction mixtures were heated in boiling water for 2 minutes and then cooled to 0°C. Substrate (^{3}H-dTMP) and products (^{3}H-dTTP/^{3}H-dDTP) were fractionated on a AG I-X8 ion exchange column (5 x 30 mm) using the following solutions for their elution in the given sequence: 2 ml H_2O, 15 ml 0.25 M ammonium formate, pH 4.3, 4 ml 4 M ammonium formate, pH 4.3. Aliquots of the eluate were dripped on glassfiber filters, dried and counted in a Beckman LS 100 liquid scintillation counter.

The counts appearing in the 4 molar wash were expressed in per cent of total counts, and specific activity expressed as per cent converted substrate per mg protein.

Separation of transcription and translation with 5-methyl-tryptophan as inhibitor of protein synthesis. The procedure was that described by Lembach et al. (26). E. coli strain NP4 was grown in 100 ml of the glycerol-casamino acid medium of Fraser and Jerrel (15) at 30°C to a density of 1 x 10^9 cells/ml. After centrifugation at 10,000 rpm for 10 minutes the pellet was resuspended in 2.5 ml of an adsorption medium, containing 0.01

molar phosphate buffer, pH 7.2, 0.4% NaCl, 0.4% KCl, 0.1% casamino acids, 0.1% glucose and 200 µg/ml 5-methyl-tryptophan. A T_4 suspension in the same medium was added to give a multiplicity of infection of 5 to 6. Two minutes after infection the cells were converted to protoplasts by adding in the following order: 2.5 ml adsorption medium, 2.5 ml 1.5 molar sucrose, 0.35 ml of 30% BSA, 0.75 ml lysozyme (2 mg/ml in Tris-HCl, pH 8.0), 0.75 ml 4% EDTA (pH 8.0), and 0.65 ml H_2O. One minute after addition of EDTA an equal volume of 0.025 molar Tris-HCl, pH 8.1 containing 0.016 molar $MgSO_4$ was added, and incubation continued for 20 minutes at 30°C. The culture was then split into two equal portions. One received 2.5 ml of a rifampicin solution (550 µg/ml in 2% ethanol); the other received the same amount of 2% ethanol. After 5 minutes for uptake of this inhibitor, protein synthesis was restored by addition of 4 volumes of a nutrient medium, containing 0.1% casamino acids, 0.1% glucose, 0.04 M $MgSO_4$, 0.3 M sucrose and 0.1 mg/ml l-tryptophan. 20 ml samples were withdrawn after 1, 10, and 20 minutes from both cultures, pelleted and resuspended in 5 ml of standard buffer. Sonication and final centrifugation were carried out as described.

Separation of transcription and translation with chloramphenicol as inhibitor of protein synthesis. The procedure employed is an alteration of the method described by Lembach et al. (26). A 100 ml culture of E. coli strain NP4 was grown on glycerol-casamino acid medium at 30°C to a density of 1×10^9 cells per ml, centrifuged and the pellet resuspended in 2.5 ml of adsorption medium without 5-methyl-tryptophan. 0.25 ml of chloramphenicol (1100 µg/ml) were added and after incubation at 30°C for 5 minutes, the culture was infected with a T_4 phage suspension (3 ml phage lysate, 0.9 ml adsorption medium, and 0.35 ml chloramphenicol) to a multiplicity of

infection of 5 to 6. After 20 minutes incubation the culture was divided into two equal portions and one half treated with 0.5 ml of a rifampicin solution (400 µg/ml dissolved in 2% ethanol). 5 minutes after addition of this inhibitor the cells were diluted into 7 volumes of ice-cold salt solution, containing 0.15 M NaCl and 0.01 M $MgCl_2$, and 4 ml of rifampicin solution or 2% ethanol were added respectively. 10 minutes later or 2 minutes respectively, the cultures were centrifuged for 10 minutes and the pellet resuspended in 5 ml ice-cold adsorption medium (without 5-methyl-tryptophan), containing 0.7 ml rifampicin or 2% ethanol. The cells were converted to protoplasts, as described before, and protein synthesis initiated by adding 4 volumes of prewarmed nutrient medium containing 0.1% casamino acids, 0.1% glucose, 0.04 molar $MgSO_4$, 0.3 molar sucrose and either 7.4 ml rifampicin solution or 2% ethanol. 20 ml samples were withdrawn after 1, 15, and 30 minutes and extracts prepared as described.

RESULTS

In an attempt to isolate unmodified host VRS from bacteriophage T_4 infected E. coli B cells in the period between phage adsorption and appearance of urea stable VRS, we found that the urea stable activity appeared earlier than expected. In a typical experiment E. coli strain NP4 was grown on Benzer Broth at 37°C and infected with bacteriophage T_4 at a multiplicity of infection (MOI) of 5-6. Aliquots were removed at 1 minute intervals, chilled with an ice-salt solution and assayed for urea stable VRS activity. As shown in Figure 1 the phage specific enzyme appeared between the first and second minute after infection and seemed to substitute for the host enzyme completely between the fifth and sixth minute.

This somewhat surprising result was not in complete agreement with the report of Chrispeels et al. of a phage specific VRS as judged by more rapid sedimentation in sucrose gradients. They suggested a time course of modification of 5 minutes for initiation and 15 to 20 minutes for completion (10). Since this work demonstrated that the phage induced conversion reaction was chloramphenicol-sensitive, we wondered whether protein synthesis was necessary for the shift to the urea stable form. E. coli strain NP4 was grown in Benzer Broth at 37°C and infected with T_4 phage. Samples removed at different times after infection were further incubated in the presence of chloramphenicol (100 μg/ml) up to 15 minutes after the time of phage addition. The result shown in Figure 2 clearly indicates that protein synthesis is required for the appearance of urea stable VRS activity.

The original work on the kinetics of the appearance of modified VRS utilized sucrose density gradient centrifugation to distinguish between the host enzyme and its phage specific high molecular weight form (10). Our

Figure 1. Appearance of urea resistant VRS activity after T_4 infection.

E. coli strain NP4 was infected with bacteriophage T_4 at time 0. 10 ml samples were withdrawn and assayed for urea stable VRS activity, correcting for the lower specific activity of the modified enzyme (see text). 100% modification refers to a recovery of 1000 CPM after treating 1000 CPM worth of enzyme with 4 M urea. Since modified enzyme appears to be somewhat activated through the urea treatment, the recovery of CPM exceeds the 100% value after 6 minutes of infection. The background was approximately 70 CPM/assay.

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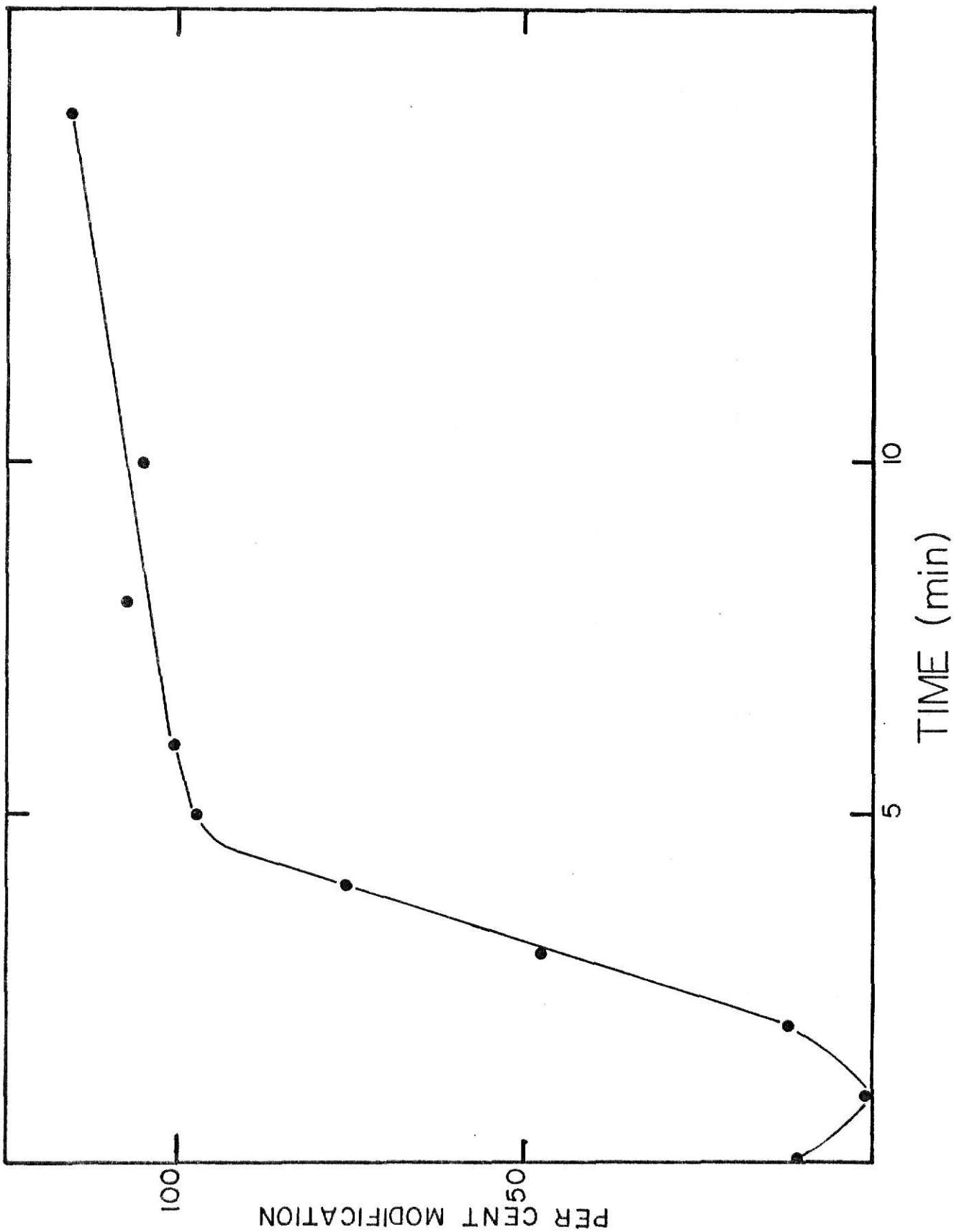
PER CENT MODIFICATION
100
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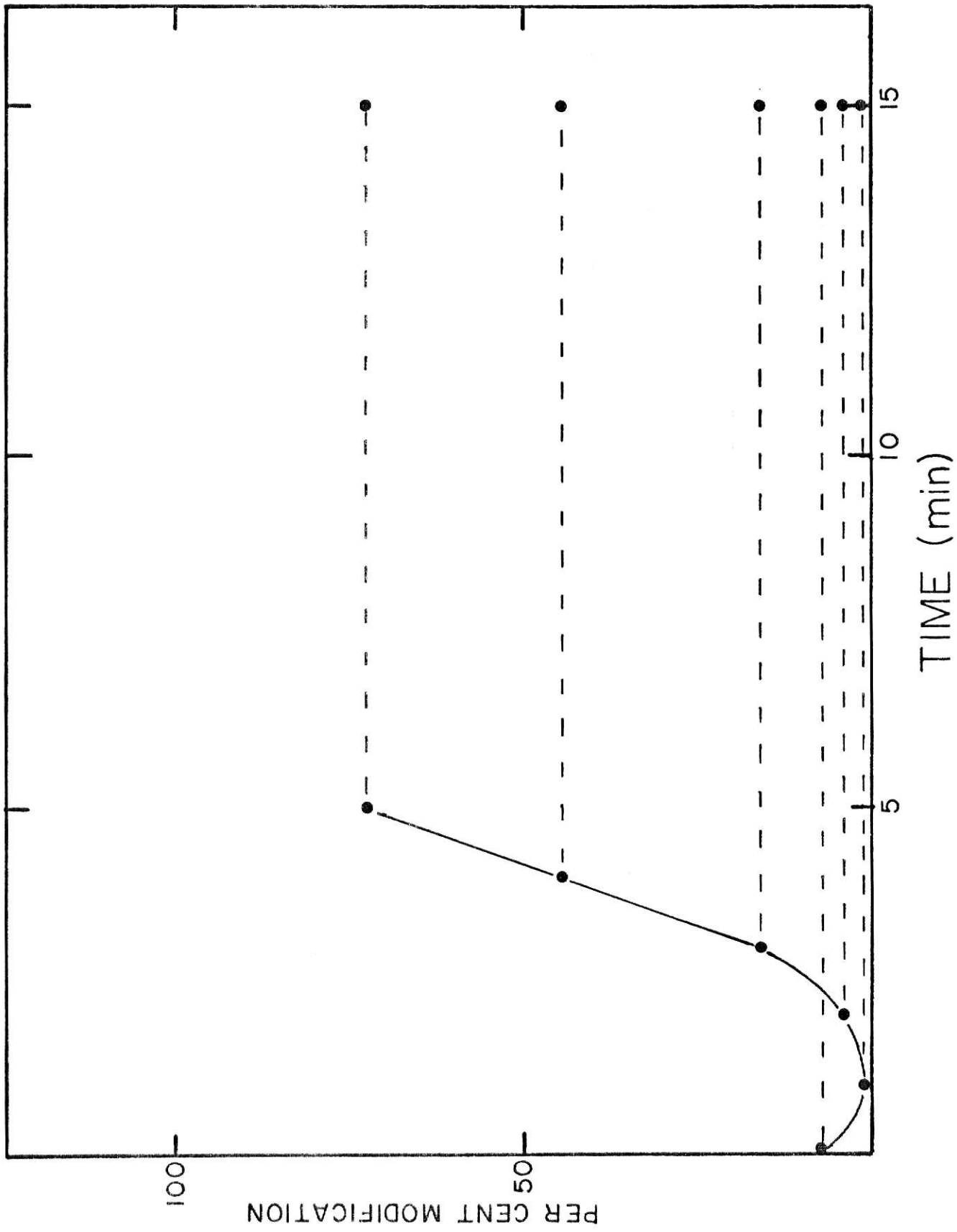
TIME (min)

Figure 2. Arrest of modification with chloramphenicol.

E. coli strain NP4 was infected with phage and 10 ml samples were withdrawn, mixed with chloramphenicol and incubated until 15 minutes after infection. The urea resistant VRS activity was plotted versus the time of withdrawing the samples to chloramphenicol.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assay for modification was the resistance of converted host VRS to treatment with 4 molar urea. Because of basic differences in these techniques we attempted to see whether there was a complete correspondence in the results derived from these procedures or whether the shift to urea stability preceded the conversion to a higher molecular weight form.

Strain NP4 was grown on Benzer Broth at 37°C, infected at time 0 with phage T_4 and samples were removed at 3, 5, 8, 10, and 15 minutes after infection. The cell extracts were tested for urea resistant VRS activity and aliquots were subjected to sucrose density gradient centrifugation. The resulting fractions were assayed for enzyme activity as well as urea stability.

Figure 3 shows that all VRS activity of the 0 minute sample is represented by a light urea sensitive peak. After 3 minutes of infection a urea stable peak appears in the heavy region, which is predominant after 5 minutes. In the 8 minute sample all activity appears to be shifted to the high molecular weight form, and no light urea unstable enzyme can be detected. Surprisingly, the urea stability of the heavy enzyme decreases with increasing time of infection, and the heavy enzyme of the 15 minute sample shows no urea resistant activity (results not shown). The complete correlation between appearing urea stability in crude extracts and rapidly sedimenting enzyme is expressed in Figure 4, where the activity of the urea stable enzyme is corrected for its lower specific activity and compared with the conversion of light to heavy VRS.

By means of both techniques, urea stability as well as sucrose density gradient centrifugation, modification of host VRS was found to be initiated at 1 to 2 minutes after infection and completed after approximately 6 minutes.

Figure 3. Sucrose density gradient centrifugation of VRS during T_4 infection.

Crude extracts of bacteriophage T_4 infected E. coli strain NP4 removed at 0, 3, 5, and 8 minutes after infection were subjected to sucrose density gradient centrifugation. VRS activity ●—● and urea stable VRS activity o—o were determined and plotted versus their relative position in the gradient.

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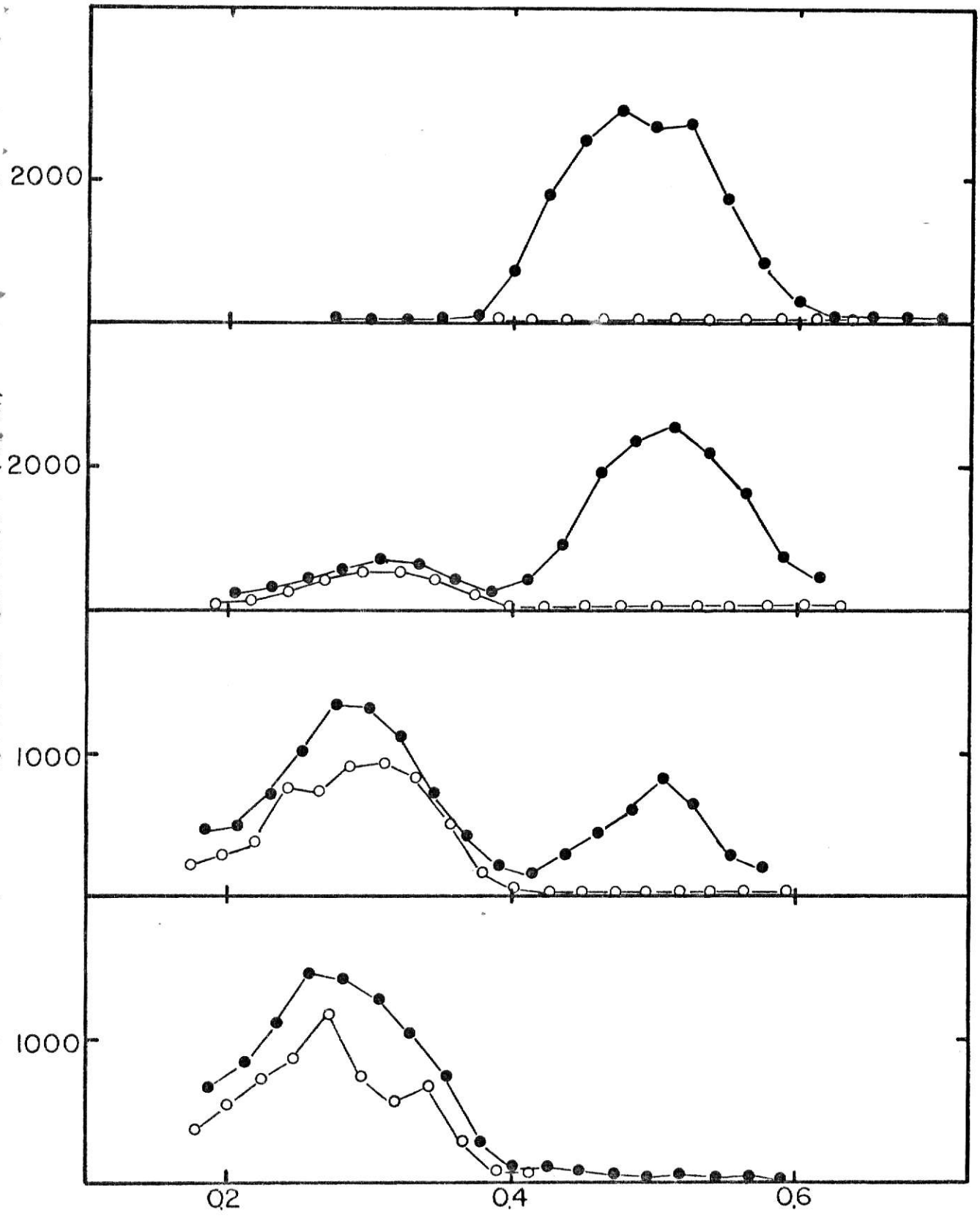
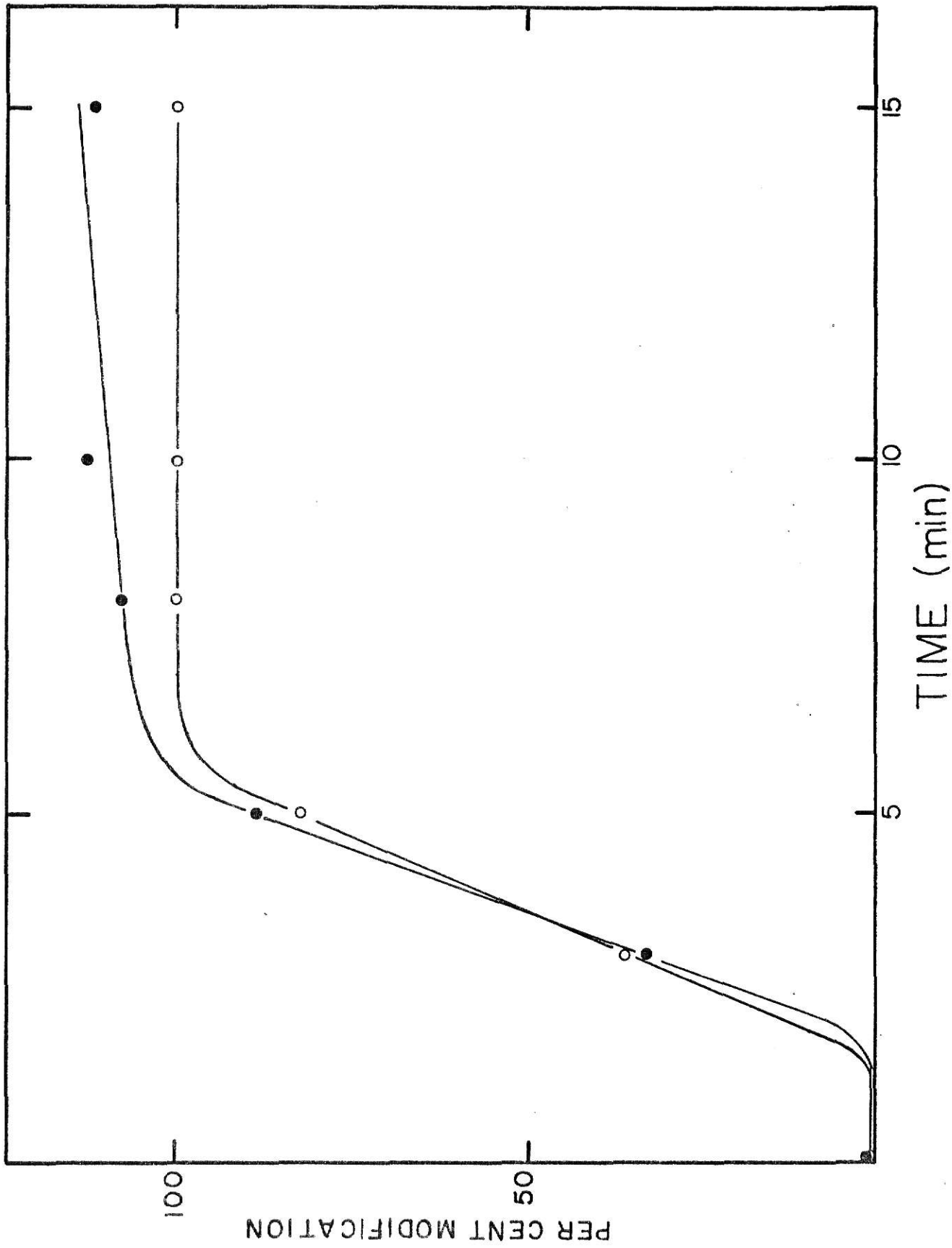

2000
2000
1000
1000
ENZYME ACTIVITY (CPM)
0.2
0.4
0.6
RELATIVE POSITION

Figure 4. A comparison of the appearance of urea stable and heavy VRS.

The same extracts subjected to sucrose density gradient centrifugation (Figure 3) were assayed for urea stability before the high speed centrifugation, and compared with the enzyme activity found in the heavy region of the sucrose gradient. ●—● per cent urea stable VRS, o—o per cent heavy VRS (both activities were corrected for their lower specific activity).


PER CENT MODIFICATION
100
50
5
10
15
TIME (min)

My findings that conversion of host VRS to the phage specific form occurs during the first few minutes after infection is in complete agreement with the corresponding work of Neidhardt *et al.* (38), who investigated the effects of bacteriophage T_4 on *E. coli* strain NP29 (I-9). This mutant possesses a temperature sensitive valyl-tRNA synthetase, which fails to charge tRNA at temperatures higher than 35°C and becomes inactive when crude extracts are prepared. After infection with phage at 30°C a new temperature resistant, stable enzyme activity appears. This phage specific activity is induced two minutes after phage addition and reaches a maximum between the ninth and twelfth minute. A different kinetic pattern of modification was suggested by Chrispeels *et al.* for *E. coli* strain NP2 (10).

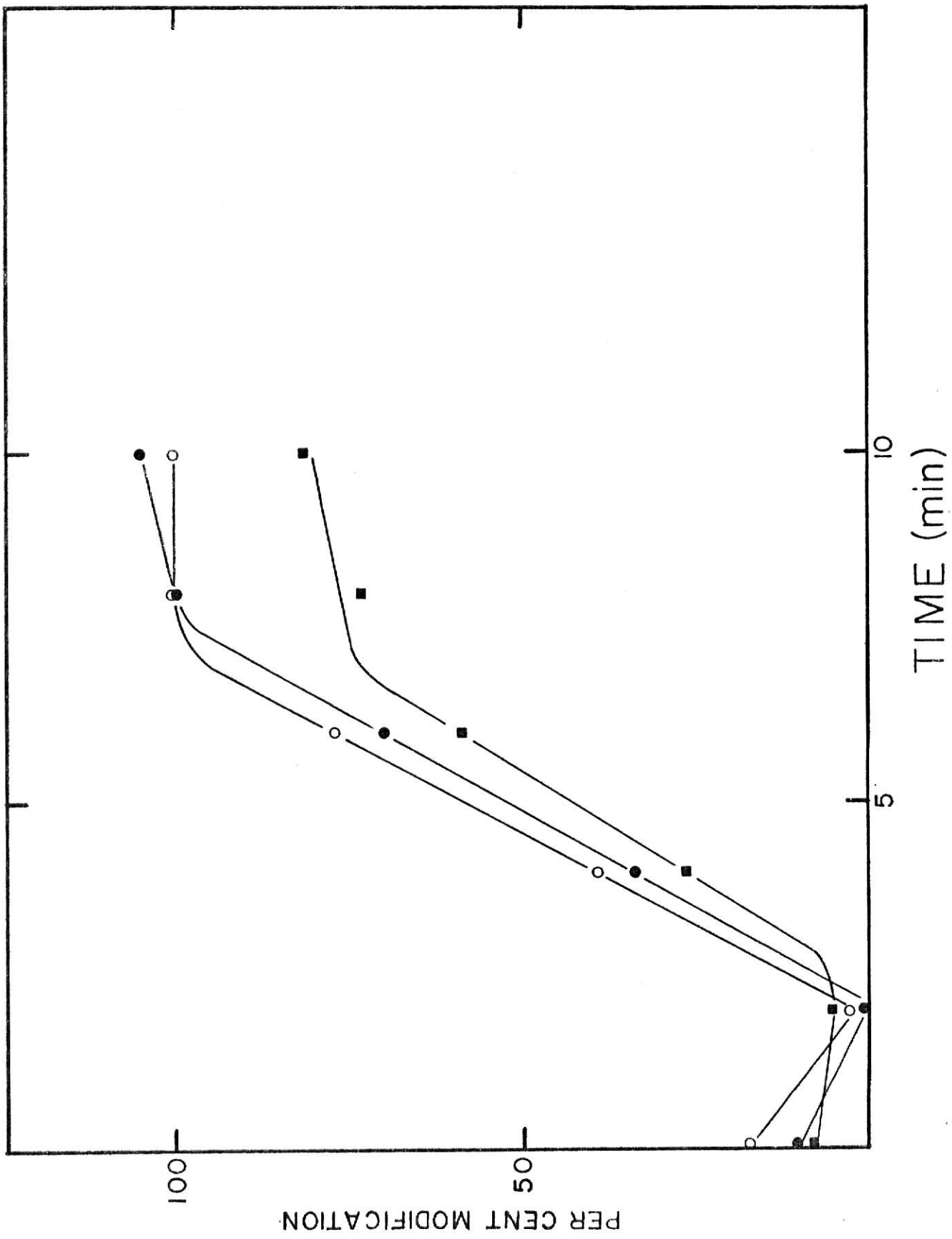
With the following experiment we attempted to analyze whether *E. coli* strain NP2, NP29, and NP4 have different time schedules for the appearance of phage specified VRS. All three strains were grown under the same conditions in casamino acid medium at 30°C, infected with T_4 and sampled at various times thereafter. Modification of host VRS was judged by urea stability in the case of NP2 and NP4 and *in vitro* activity in the case of NP29.

The results shown in Figure 5 indicate that regardless of the strain used the phage induced enzyme activity appears after 2 minutes and reaches its maximum value between 7 and 8 minutes after infection.

Enzymatic and chemical reactions are known to respond to changes in temperature with a corresponding change in reaction velocity. The effect of temperature on the onset and velocity of VRS modification was determined in a series of experiments where two cultures of *E. coli* NP4 were grown under the same conditions on either Benzer Broth or M9-minimal media. The temperature of growth and infection was varied from 10° to 37°C in different experiments.

Figure 5. Appearance of modified VRS in several E. coli strains.

E. coli strains NP2, NP4 and NP29 were infected with T_4 and assayed for modified VRS activity (urea stable VRS in NP2 and NP4 and stable in vitro VRS activity in the case of the temperature sensitive mutant NP29). The activity of the 8 minute extract was termed 100%. ●——● NP4 urea stable VRS, ■——■ NP2 urea stable VRS, o——o NP29 VRS activity.


PER CENT MODIFICATION
100
50
5
10
TIME (min)

Before infecting the cells with bacteriophage T_4 the generation time of each culture was determined. With the normal procedure samples were removed at various stages in the infection cycle, protein synthesis stopped with chloramphenicol and the crude extracts assayed for urea stable VRS.

The relation between absolute temperature and specific growth rate constant (K) is expressed in an Arrhenius plot (Figure 6a). Plotting the logarithm of K versus the reciprocal value of T, (1/Kelvin), gives a straight line for the temperature range of 20° to 37°C. From the slope of these lines it can be calculated that a temperature increase of 10°C results in an approximately 2.5 fold increase in growth rate for both the Benzer Broth and the M9 culture (Q_{10} = 2.5).

A similar plot for the modification reaction is shown in Figure 6b. The slope of the urea stability curve of each culture was independently determined and used as the parameter for the velocity (v) of VRS modification. In the case of the M9 culture the plot of log v versus 1/T reveals that the phage induced modification of E. coli VRS follows the Arrhenius equation when growth and infection is carried out in the range of "physiological" temperatures (20°-37°C). The Q_{10} for this reaction was calculated to be 2.7, which is in the range expected for chemical reactions. The same Q_{10} value was found for the Benzer Broth culture at temperatures between 20° and 30°C. At 37°C, however, the velocity for the modification did not quite reach its expected value and in several experiments did not exceed the value found for the M9 culture at this temperature.

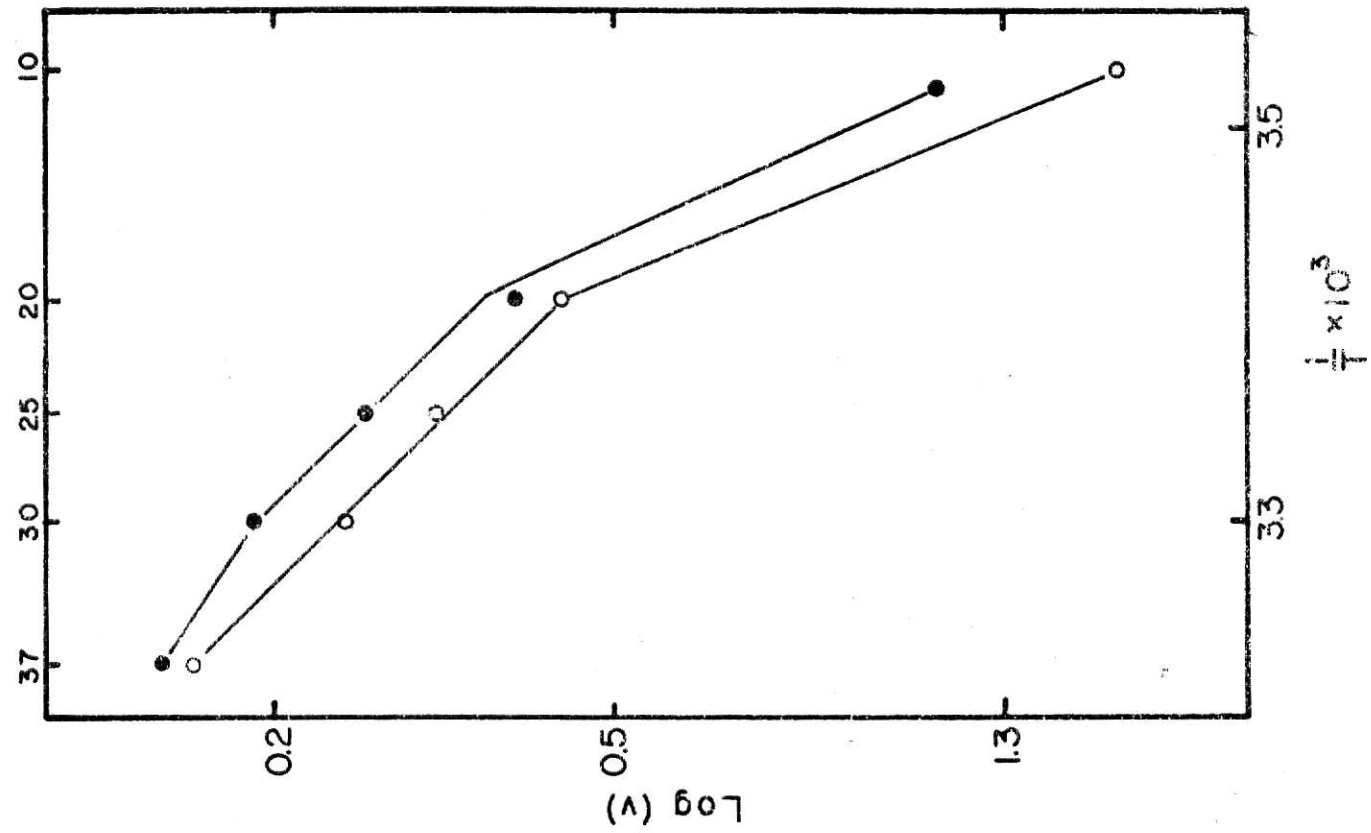
While the rate of the appearance of urea stable VRS can be obtained, an accurate determination of the onset of this reaction is somewhat more difficult. During the first few minutes (depending on the temperature) the

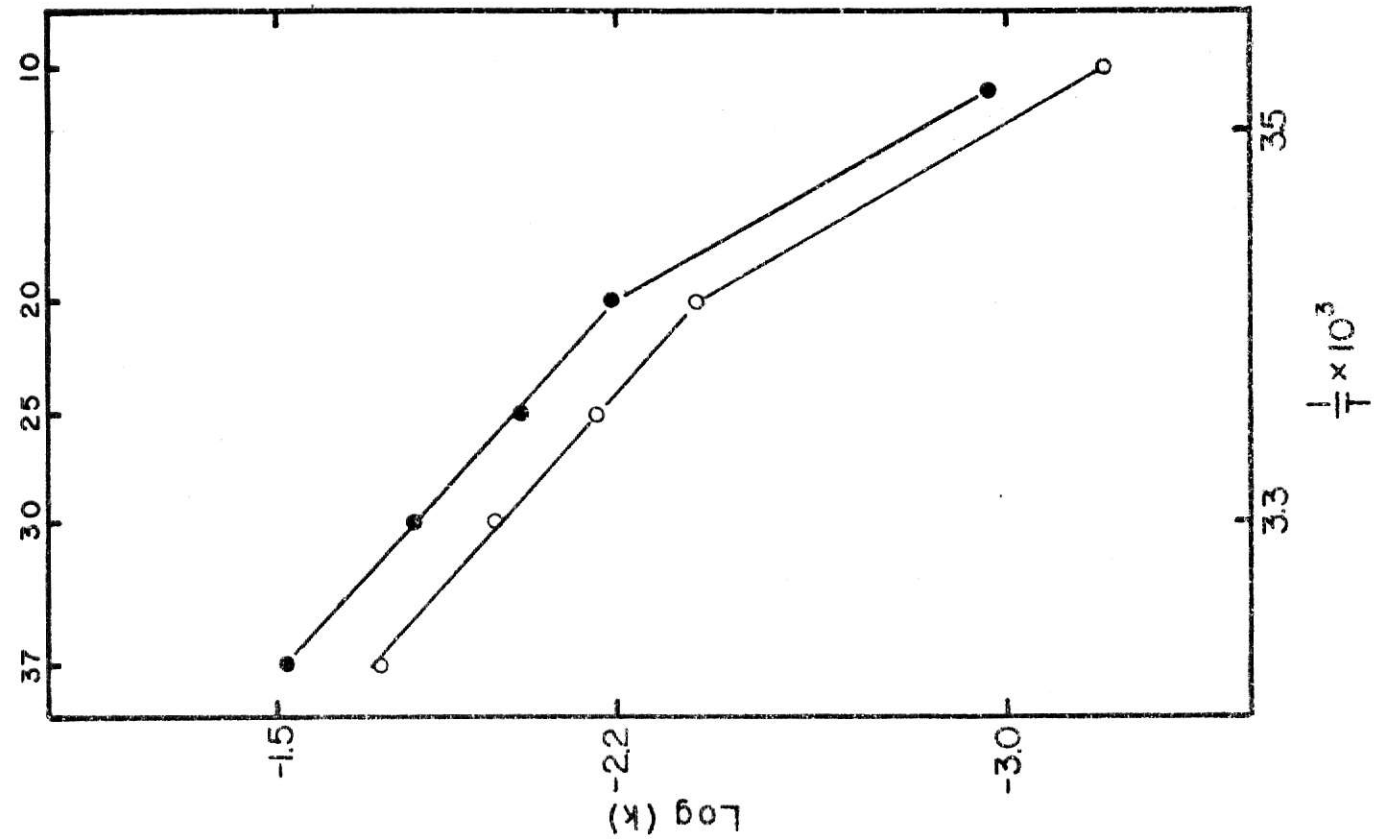
Figure 6a. Arrhenius plot of growth rates in rich and minimal media.

E. coli strain NP4 was grown on Benzer Broth ●—● and M9 minimal media o—o at different temperatures, and the growth rate constant K was determined.

Figure 6b. Arrhenius plot of the velocities of modification in rich and minimal media.

The cultures described in Figure 6a were infected with T_4 and the velocity of modification was determined. Benzer Broth culture ●—●, M9 culture o—o .


10
20
25
30
37
0.2
0.5
1.3
Log (v)
3.5
3.3
$\frac{1}{T} \times 10^3$


10
20
25
30
37
-1.5
-2.2
-3.0
Log (k)
3.5
3.3
$\frac{1}{T} \times 10^3$

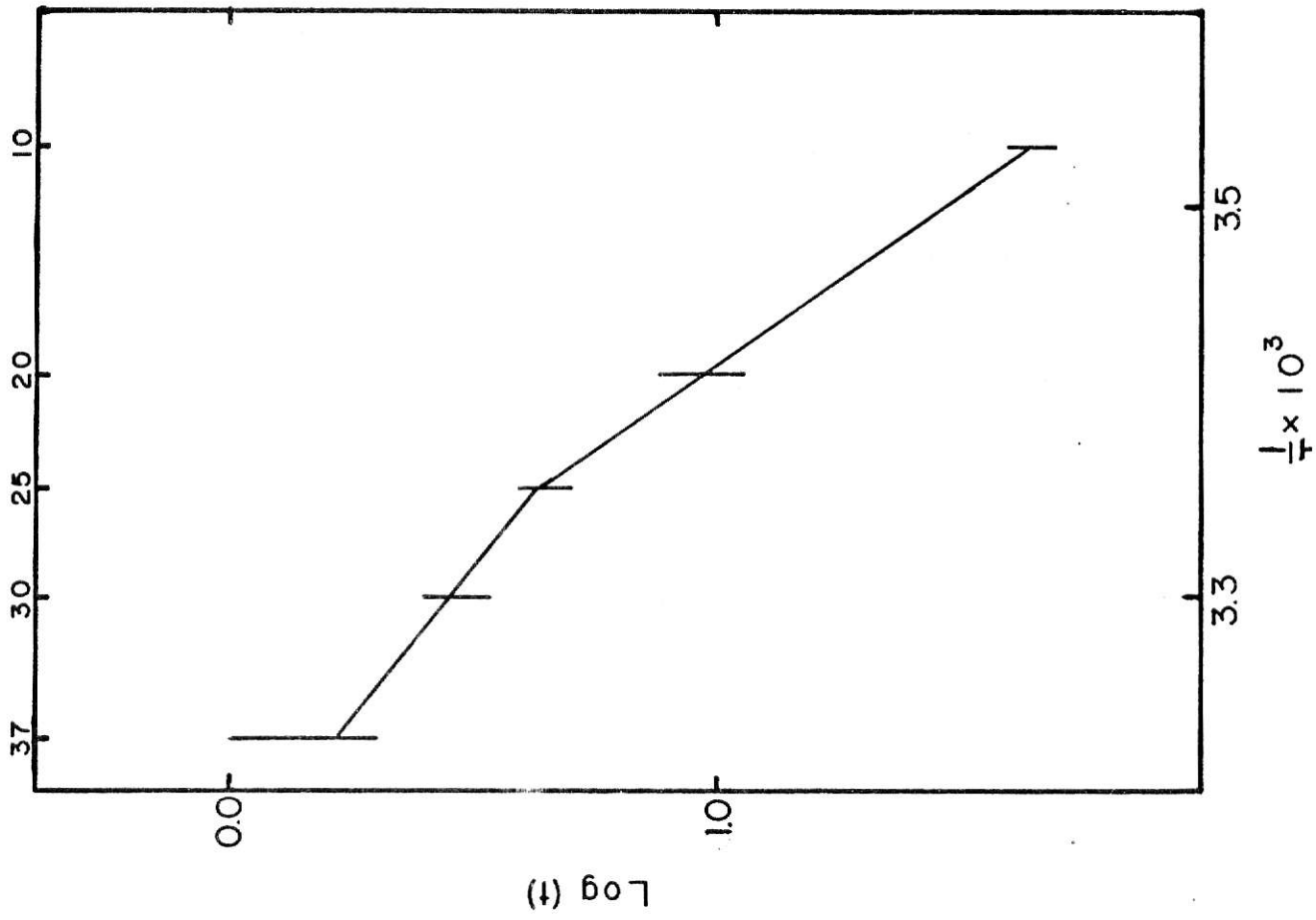
reaction appears to be nonlinear; for this reason the determination of the time of initiation was subject to larger variation.

Although not much is known about the exact mechanism of modification, it is obvious that several complex biochemical reactions are involved in the synthesis of the modifying factor $\underline{\tau}$. It is reasonable to assume that an expression of the overall velocity of these reactions can be the time at which enough $\underline{\tau}$ is produced to initiate the modification of host VRS. With this approach the effect of temperature on the onset of modification can be expressed in an Arrhenius plot shown in Figure 6c. The straight line obtained for temperatures between 10° and 25°C indicates that the initiation time is inversely proportional to the absolute temperature. The Q_{10} value for this part of the curve was found to be 4.7 (maximal variation 4-5.6), indicating that with a temperature increase of 10°C the initiation time decreases to one-fifth of its original value. This appears not to be true for temperatures between 25 and 37°C since the curve levels off at 25°C. The corresponding Q_{10} value of approximately 2.2 (maximal variation 1.74-3.55) suggests that the influence of temperature on the onset of modification is definitely reduced above 25°C.

The data described above also indicate the influence of the carbon source on the onset and velocity of modification. For each experiment both cultures were grown under identical conditions with the medium as only variable. Figure 6a shows that the growth curves for both cultures in an Arrhenius plot are nearly parallel over the total temperature range from 10° to 37°C. Therefore preferential effects of the temperature on the growth rate of one of the cultures can be excluded. Thus the slope and level of these curves is only a function of energy source, indicating that the

Figure 6c. Arrhenius plot of the onset of modification in rich and minimal media.

The cultures described in Figure 6a were infected with T_4 and the onset of modification was determined. Due to larger variation in determining the exact time (t) of onset of modification, the log (t) is expressed as a range rather than by points. Each line represents the extend of maximal variation for both the M9 and Benzer Broth culture.


10
20
25
30
37
0.0
1.0
Log (t)
3.5
3.3
$\frac{1}{T} \times 10^3$

velocity of growth is dependent on the media but not the Q_{10} of this process. Similar results are obtained for the velocity of modification (Figure 6b). Between 10° and 30°C the Arrhenius plots are parallel and only the magnitude of velocity seems to be a function of the media. An identical Q_{10} value for both cultures suggests that the change in velocity is independent upon the energy source but directly related to the temperature of growth.

A media effect can be observed at temperatures above 30°C, where the Q_{10} of the M9 culture is still constant, the corresponding value for the Benzer Broth culture, however, decreases to approximately 1.9. This suggests that both the temperature and the media influence the change in reaction velocity. A possible explanation for this effect will be given in the discussion.

The correlation between medium and onset of modification is shown in Figure 6c. The striking difference in growth rate and reaction velocity due to different energy sources is not reflected in the time at which the modification reaction is initiated. Thus the values of both cultures can be represented by one line. This indicates that the process responsible for modification of host VRS is initiated independent from the growth rate but in proportional relationship to the temperature.

In 1966 Neidhardt *et al.* suggested that the time course of the change in valyl-tRNA synthetase activity in the mutant *E. coli* strain NP29 infected with T_4 phage is roughly similar to the development of T_4-induced deoxycytidylate deaminase (dCMP-deaminase) activity (39). Since we could show that this seemed to be true for wildtype *E. coli B* and *K* strains infected with T_4 phage, we wondered how the appearance of phage specific VRS fit into the pattern of early enzyme synthesis when compared with three other early enzymes.

Dihydrofolic acid reductase (FH_2 reductase) was found by Mathews and Cohen to be increased several fold after T_6 infection of E. coli cells (33) and shown to be an "early" enzyme by several other laboratories (26, 32, 55, 56).

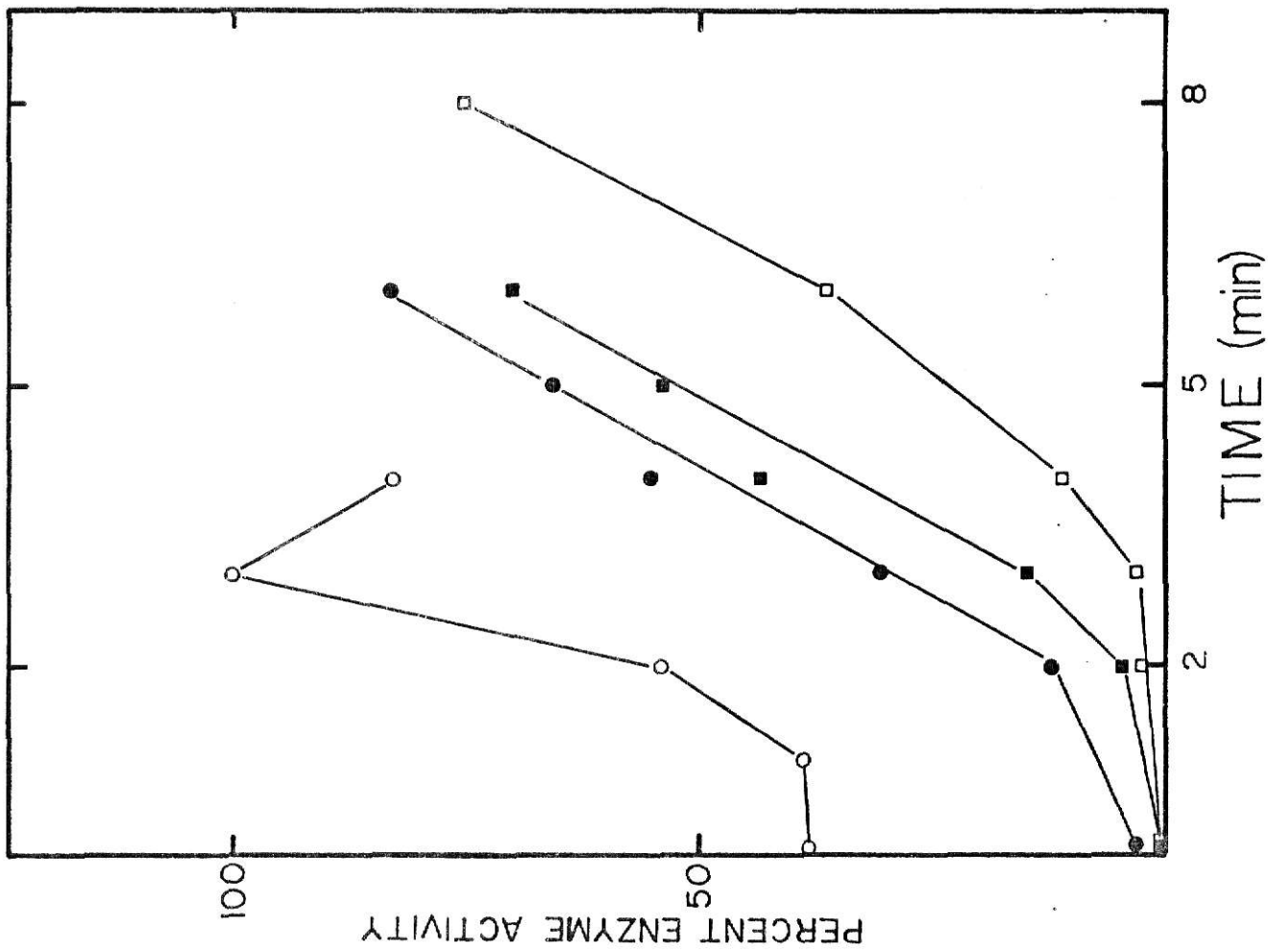
Keck et al. detected the induction of dCMP deaminase in E. coli 3 to 5 minutes after infection with T_2 bacteriophage (56). Because of its involvement in DNA synthesis and the time of its appearance it was defined to be "early" (32, 55).

Bessman investigated the change in deoxyribonucleotide kinase (dTMP kinase) in T_2, T_4, and T_6 infected E. coli cells and showed that the enzyme level is increased several fold between 3 and 10 minutes after infection with T_4 (5). This enzyme was also shown to be an early phage function (26, 32, 44).

Because of their differential synthesis (26, 56) we chose these enzymes for a comparison with the phage induced modification of E. coli VRS. A Benzer Broth culture of E. coli strain NP4 was infected with T_4 phage at 37°C and samples withdrawn at various times after infection were assayed for the activity of FH_2 reductase, dCMP deaminase, dTMP kinase and urea stable VRS. Figure 7 shows a plot of enzyme activity versus time of infection. Since only the first 8 minutes were monitored for the appearance of these enzymes, and the dCMP deaminase and dTMP kinase did not reach their maximal activity during this period, a correct comparison of enzyme level in terms of per cent activity would be rather difficult. In order to be able to compare these enzymes with the two other activities, the dTMP kinase activity in the 8 minute sample was defined to be 75% (compare ref. 5) and the dCMP deaminase level in the 6 minute extract was assumed to be 70% (compare 24, 55, 56).

Figure 7. Comparison of T_4 specific "early" enzymes.

E. coli strain NP4 was grown at 37°C in Benzer Broth, infected with bacteriophage T_4 at time 0, and assayed at various times after infection for urea stable VRS activity ●—●, dCMP deaminase activity ■—■, FH_2 reductase activity ○—○ and dTMP kinase activity □—□. Per cent activity (see text) was plotted versus time after infection.


PERCENT ENZYME ACTIVITY
100
50
TIME (min)
2
5
8

The FH_2 reductase appeared to reach its 100% value at about 3 minutes after infection, and the phage specific VRS activity was expressed in per cent urea stability as described under materials and methods. Under these conditions a different kinetic order of synthesis for the individual enzymes was observed. The dihydrofolic acid reductase was expressed after the first minute of infection and reached its maximum level at a time where the dTMP kinase activity was barely detectable. The dCMP deaminase expression preceded the kinase by approximately one minute but was not detectable before the second minute of infection. Urea stable VRS activity preceded the deaminase activity by nearly one minute. Although DNA synthesis was not halted under these conditions, the kinetic similarities between VRS modification and dCMP deaminase expression framed by two other early enzymes suggests that conferral of urea stability on VRS is an early function of bacteriophage T_4.

Using 5-methyl-tryptophan and rifamycin as inhibitors of protein and RNA synthesis respectively, Lembach and Buchanan were able to separate the early transcriptive events of bacteriophage infected E. coli cells into a period of transcription and one of translation (26). Thus they proposed three classes of early enzymes with FH_2 reductase belonging to the first and deoxinucleotide kinase belonging to the third class. The messenger for class I enzymes was found to be transcribed in the presence of 5-methyl-tryptophan, the class III mRNA did not accumulate under these conditions. Class II messenger was transcribed to an intermediate degree. With a similar system we tried to further characterize the timing of vs gene expression by defining its product ɪ to be either "immediate" or "delayed early."

E. coli strain NP4 was grown on glycerol-casamino acid medium, shifted to a medium containing 5-methyl-tryptophan and infected with bacteriophage T_4.

After conversion of the infected cells to protoplasts they were incubated for 20 minutes at 30°C to inhibit protein synthesis, divided into two equal portions and rifampicin was added to one. After five minutes for uptake of this rifamycin-derivative further mRNA synthesis was arrested; protein synthesis was restored by the addition of tryptophan containing growth medium. Samples of both cultures were assayed for urea stable VRS, FH_2 reductase and dTMP kinase activity.

The enzyme levels found in the 20 minute sample of the culture that did not receive rifampicin were defined to be 100%. Our results shown in Figure 8 match the findings of Lembach and Buchanan. FH_2 reductase appeared to be synthesized to a considerable extent in the presence of 5-methyl-tryptophan; yet, an increase in activity after restoration of protein synthesis indicated that the accumulation of its messenger was not rifampicin sensitive. The opposite result was found for the dTMP kinase activity, which increased several fold in the translation period only when mRNA synthesis was not simultaneously arrested. The pattern of urea stable VRS activity matched very closely the behavior of the reductase. Although modification occurred in the presence of the tryptophan derivative, an increase from 60 to 85% in absence of concomitant mRNA synthesis suggested that a considerable amount of $\underline{\tau}$ specific messenger was transcribed under conditions, which did not allow the transcription of the dTMP kinase gene. The real increase in modification in this case was 43.5 to 61.2 per cent.

The preceding experiment revealed that 5-methyl-tryptophan does not shut off protein synthesis completely and allows the production of at least some proteins. Therefore chloramphenicol, known to block protein synthesis completely, was used in a second experiment. The procedure was similar to

Figure 8. Early transcriptive events in the presence of 5-methyl-tryptophan.

E. coli strain NP4 was grown on glycerol-casamino acid medium at 30°C and infected with bacteriophage T_4 in the presence of 5-methyl-tryptophan. After arresting mRNA synthesis with rifampicin in one culture, protein synthesis was restored by the addition of l-tryptophan. Extracts prepared at various times after restoration of protein synthesis were assayed for the following enzyme activities:

●—● urea stable VRS activity in the control culture,

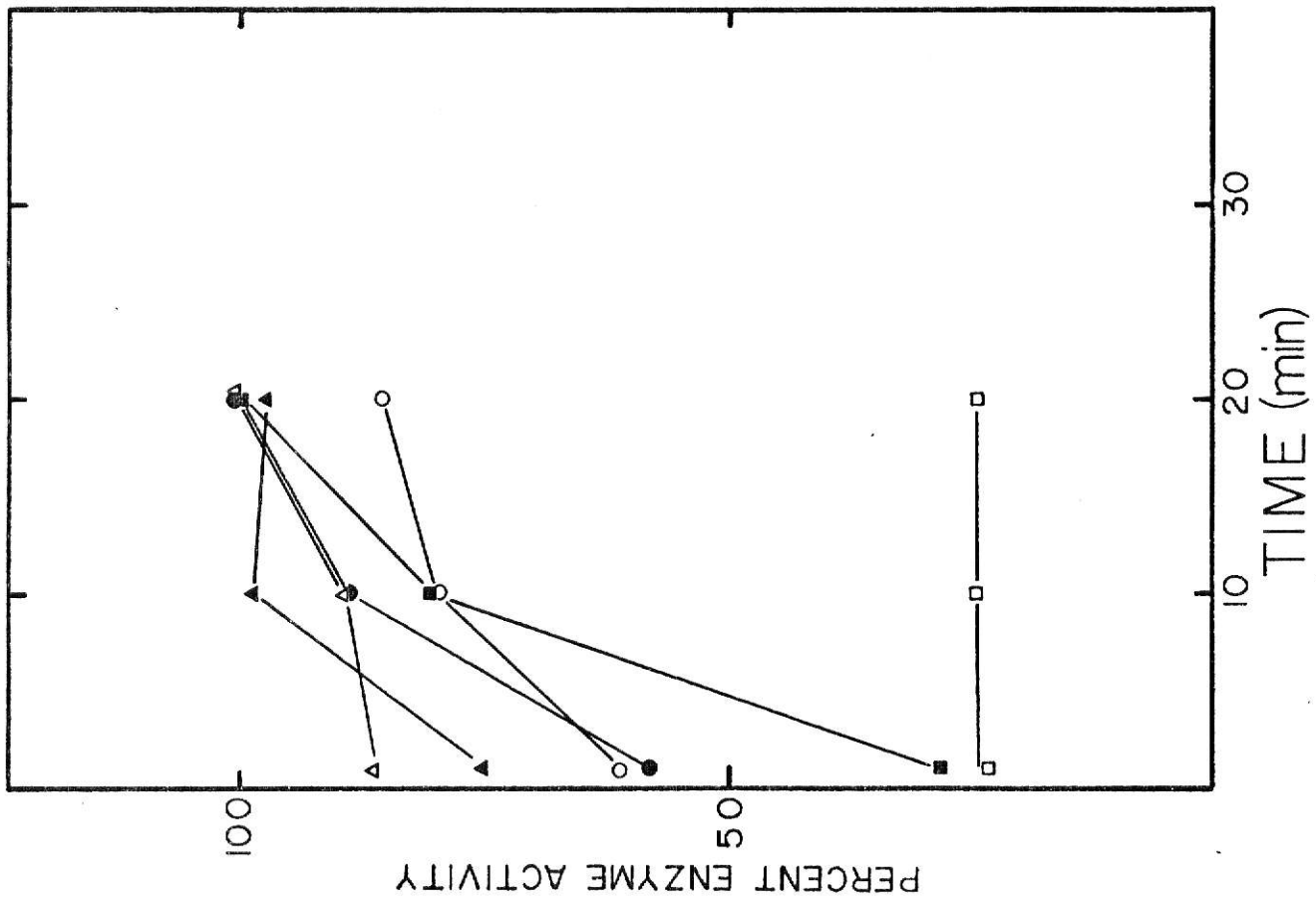
○—○ urea stable VRS activity in the rifampicin culture,

▲—▲ FH_2 reductase activity in the control culture,

△—△ FH_2 reductase activity in the rifampicin culture,

■—■ dTMP kinase activity in the control culture,

□—□ dTMP kinase activity in the rifampicin culture.


PERCENT ENZYME ACTIVITY
100
50
10
20
30
TIME (min)

the 5-methyl-tryptophan experiment and is described in detail under materials and methods. A disadvantage of chloramphenicol is the time required for diluting out the inhibitor before protein synthesis can be restored, because of the short half-life time of mRNA. When the time between diluting the cells with the ice-cold salt medium and centrifugation was chosen to be 10 minutes the result shown in Figures 9a and 9b was obtained. While FH_2 reductase and VRS modifying factor $\underline{\tau}$ were readily expressed even when chloramphenicol was present during the period of transcription, no kinase activity was found in the corresponding extracts. The dCMP deaminase activity seemed to be transcribed also in the presence of chloramphenicol, which might reflect an experimental error however, because of the very low activity of this enzyme in the control culture.

In a second experiment the cultures were kept in the ice-cold salt solution only two minutes before centrifugation to decrease the extent of mRNA degradation. Figures 10a and 10b reveal an increase of FH_2 reductase and modified VRS activity in the rifampicin cultures as compared to the control extracts, but no such effects were observed for the dTMP kinase. The dCMP deaminase activity seemed not to be transcribed in the presence of the inhibitor under these conditions.

These results suggest that the phage specific $\underline{vs}$ gene is transcribed in the absence of concomitant protein synthesis and the ability at least in part to produce urea stable VRS is therefore an "immediate early" function of bacteriophage T_4.

Only a few phage induced enzymes are known as immediate early, and most of them are directly involved in the synthesis of phage specific DNA. Thus they fulfill essential functions in the early development of active phages,

Figure 9. Early transcriptive events in the presence of chloramphenicol.

E. coli strain NP4 was grown on glycerol-casamino acid medium at 30°C and infected in the presence of chloramphenicol. After addition of rifampicin to one culture the chloramphenicol was diluted out of both cultures for 10 minutes and protein synthesis was restored. Both cultures were assayed for enzyme activity:

Figure 9a)

● —— ● urea stable VRS in the control culture

○ —— ○ urea stable VRS in the rifampicin culture

■ —— ■ FH_2 reductase in the control culture

□ —— □ FH_2 reductase in the rifampicin culture

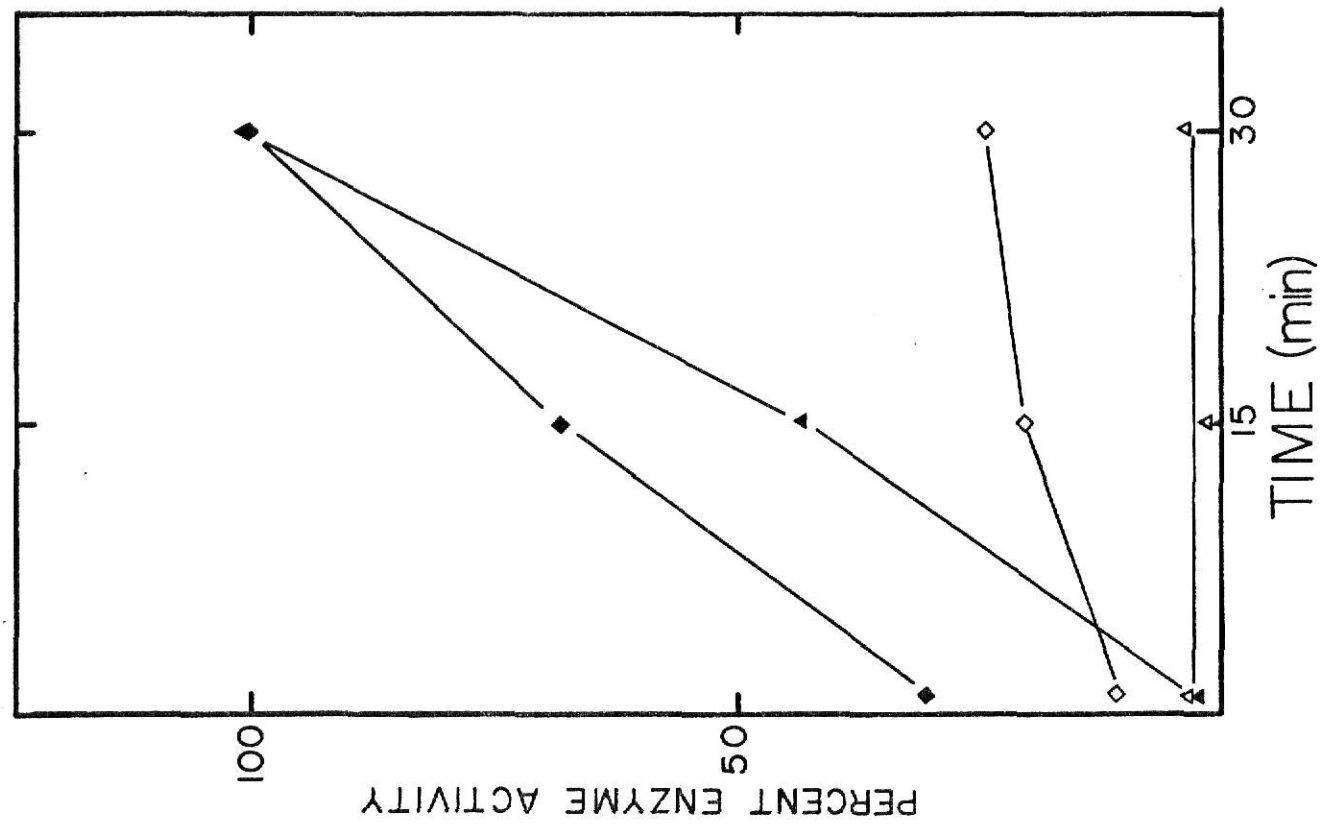
Figure 9b)

◆ —— ◆ dCMP deaminase in the control culture

◇ —— ◇ dCMP deaminase in the rifampicin culture

▲ —— ▲ dTMP kinase in the control culture

△ —— △ dTMP kinase in the rifampicin culture


PERCENT ENZYME ACTIVITY
100
50
15
30
TIME (min)

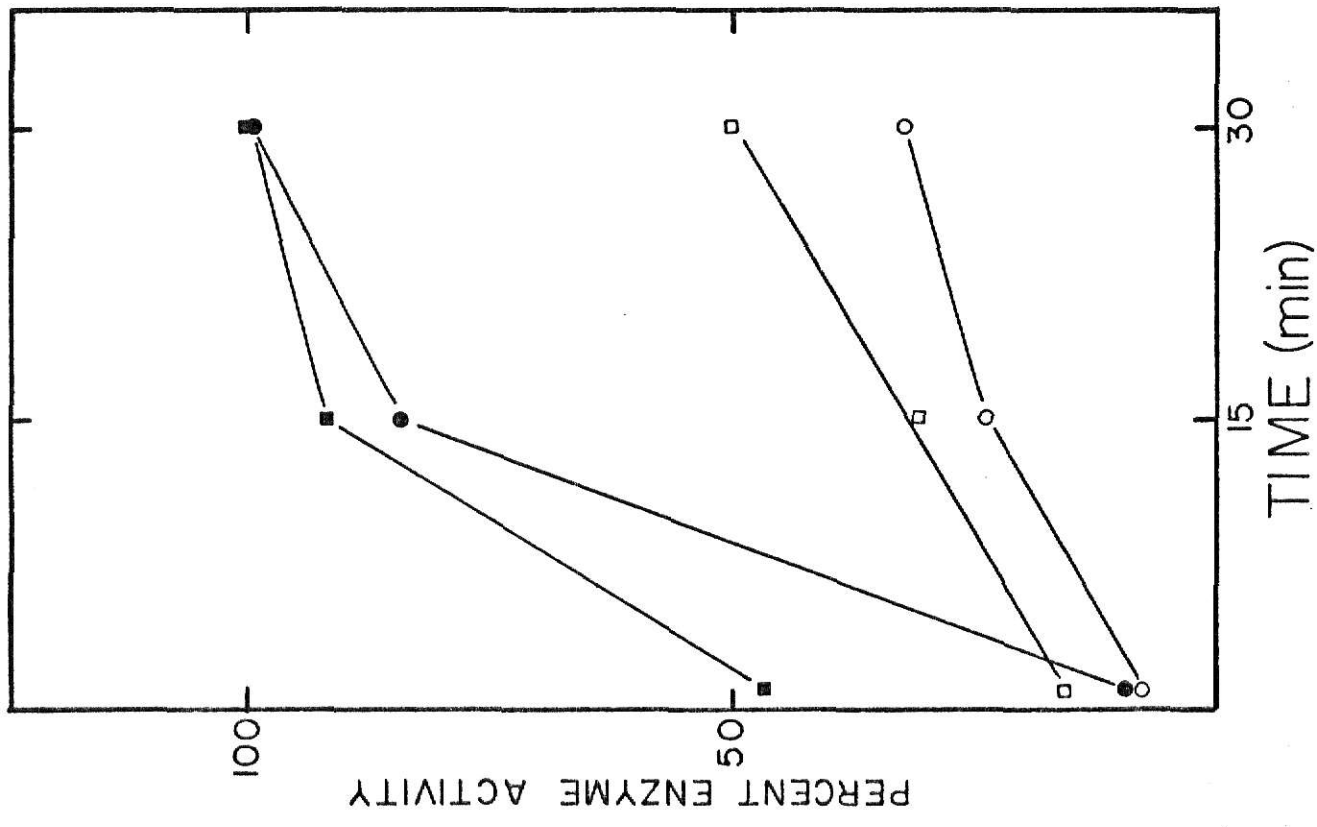

PERCENT ENZYME ACTIVITY
100
50
15
30
TIME (min)

Figure 10. Early transcriptive events in the presence of chloramphenicol.

The same experiment as described under Figure 10 was performed except that the time for diluting out the chloramphenicol was reduced to 2 minutes:

Figure 10a)

●—● urea stable VRS in the control culture,

○—○ urea stable VRS in the rifampicin culture,

■—■ FH_2 reductase in the control culture,

□—□ FH_2 reductase in the rifampicin culture,

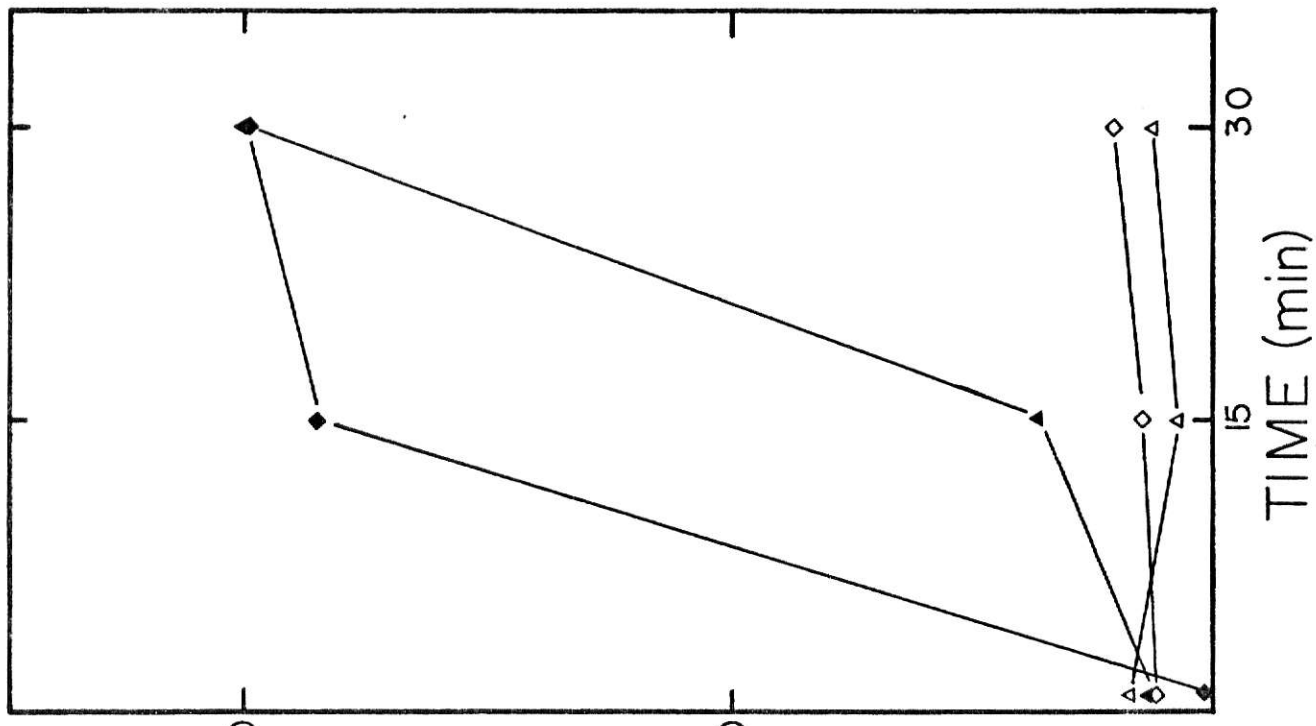
Figure 10b)

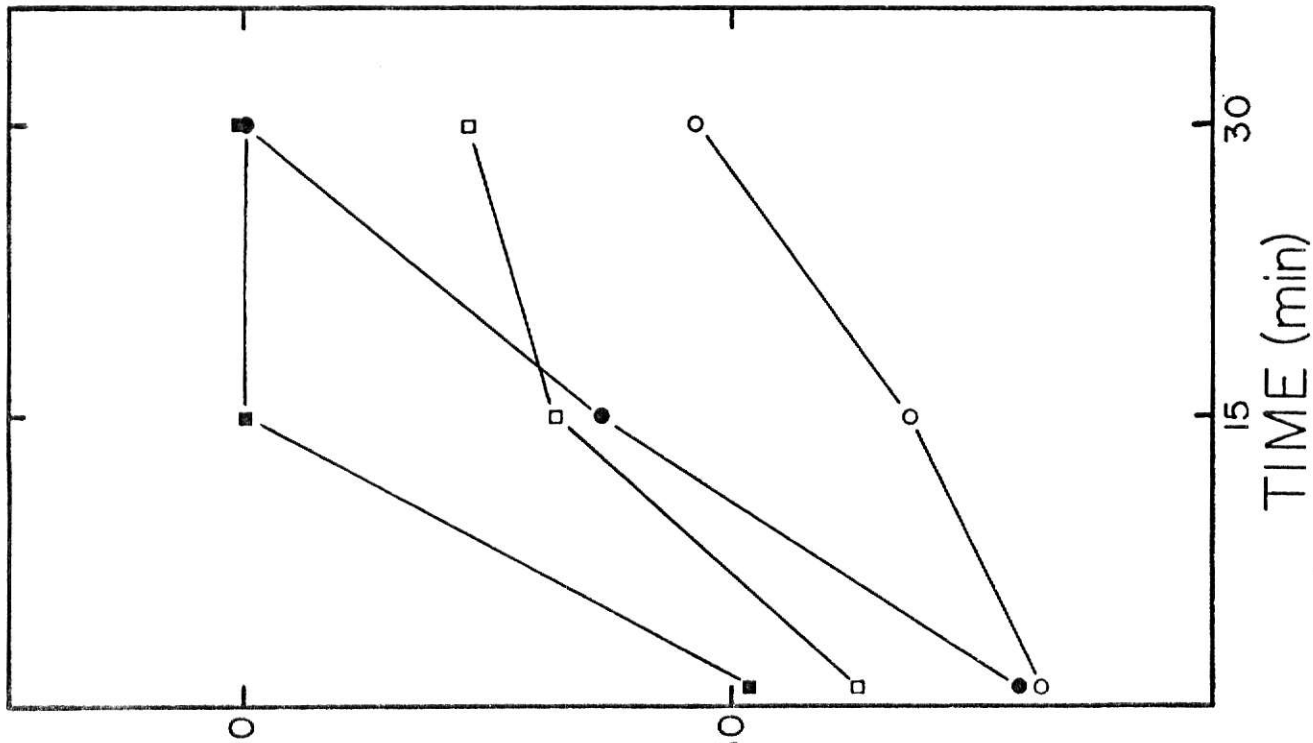
◆—◆ dCMP deaminase in the control culture,

◇—◇ dCMP deaminase in the rifampicin culture,

▲—▲ dTMP kinase in the control culture,

△—△ dTMP kinase in the rifampicin culture.


PERCENT ENZYME ACTIVITY
100
50
15
30
TIME (min)


PERCENT ENZYME ACTIVITY
100
50
15
30
TIME (min)

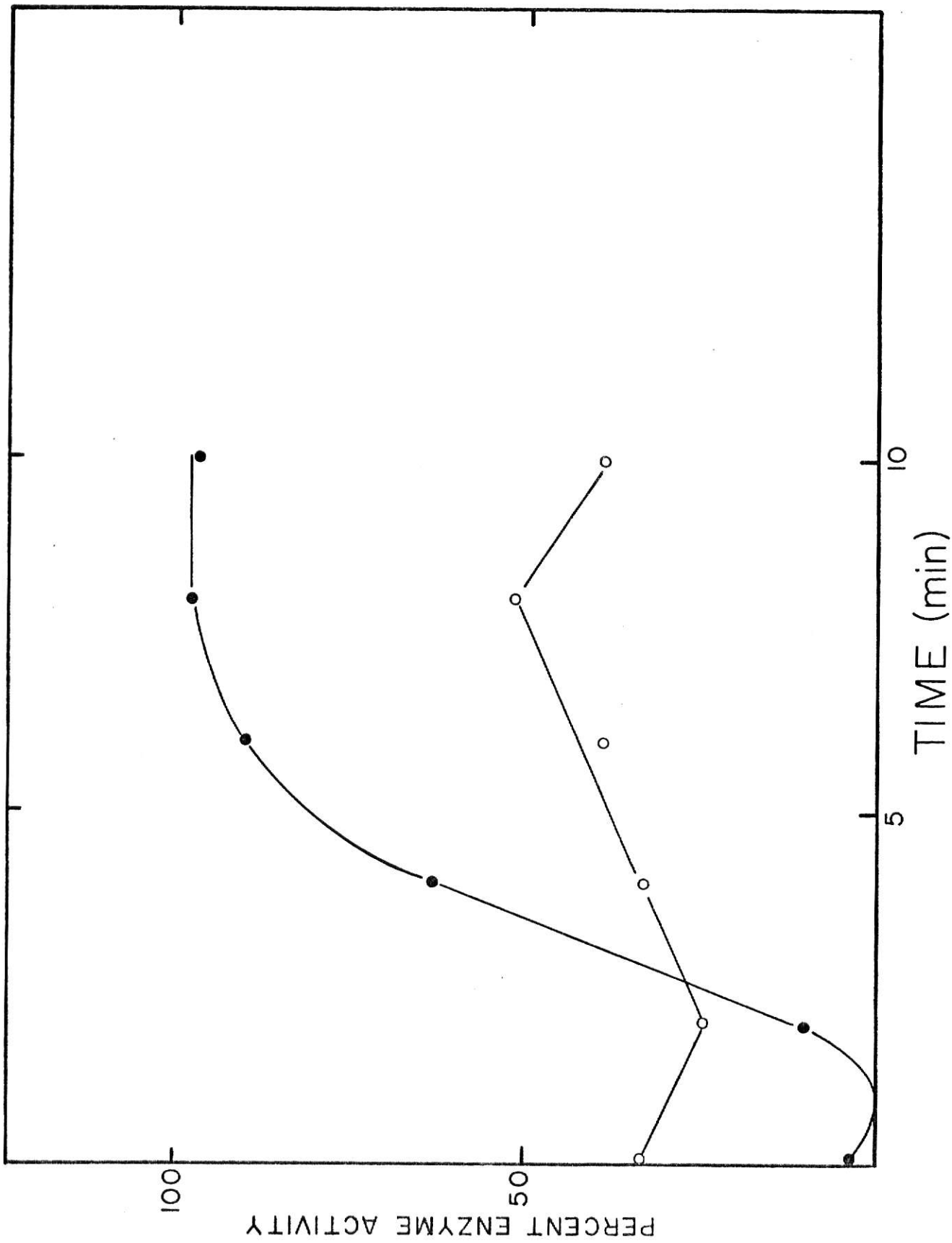
which explains the necessity of their immediate synthesis. Viewed under this aspect, the immediate appearance of urea stable VRS suggests a real need for such an early phage function. However, T_4 mutants incapable of producing urea stable VRS activity were still able to produce normal burst sizes when cultivated under normal laboratory conditions. Since the _I_ peptide is at least in part responsible for urea stability, this argues that either _I_ is essential and absolute negative mutants represent a lethal situation, or that mutants isolated so far produced enough of the peptide to be physiologically active but undetectable by the criterion of urea stability.

The former question might be pursued by attempting to isolate conditional _vs_ mutants, the latter awaits the development of a _I_ assay for possible detection of peptide fragments. In the short term several guesses on the physiological significance of modification were investigated.

The work of Puck and Lee (41, 42) and Guttman _et al._ (21) indicated a change in the magnesium ion concentration within the cell after bacteriophage infection. The magnesium dependence of the aminoacyl-tRNA synthetase reactions led us to think of a possible interference of VRS modification with a sudden drop in the concentration of these ions. An attachment assay with cell extracts removed at various times after infection with bacteriophage T_4 was performed, using a 10, 100, and 1000 times lower magnesium concentration than usual. While at a Mg^{++} concentration of 10^{-4} and 10^{-5} M the VRS activity dropped below 10% of the usual rate, considerable active enzyme was obtained with 10^{-3} M Mg^{++}. As shown in Figure 11 the wildtype enzyme reacts with approximately one-third of its normal activity under these reaction conditions. After 2 minutes of infection the recovery is just 25%, but increases to 52% of the normal level at 8 minutes after infection.

Figure 11. VRS activity at low Mg^{2+} ion concentration.

E. coli strain NP4 was infected with bacteriophage T_4 and extracts were prepared at various times after infection. An attachment assay with 10 fold lower Mg^{2+} ion concentration (10^{-3} M) than usual was performed and the recovery of activity in per cent compared with the per cent urea stability (corrected). ● — ● urea stable VRS activity, o — o per cent VRS activity with 10^{-3} M Mg^{2+}.


PERCENT ENZYME ACTIVITY
100
50
5
10
TIME (min)

Since in cell free extracts the specific activity of the wildtype enzyme is approximately two fold higher than the activity of the modified form, the simultaneous increase of modification and enzyme activity at low magnesium concentration is probably insignificant for the in vivo conditions.

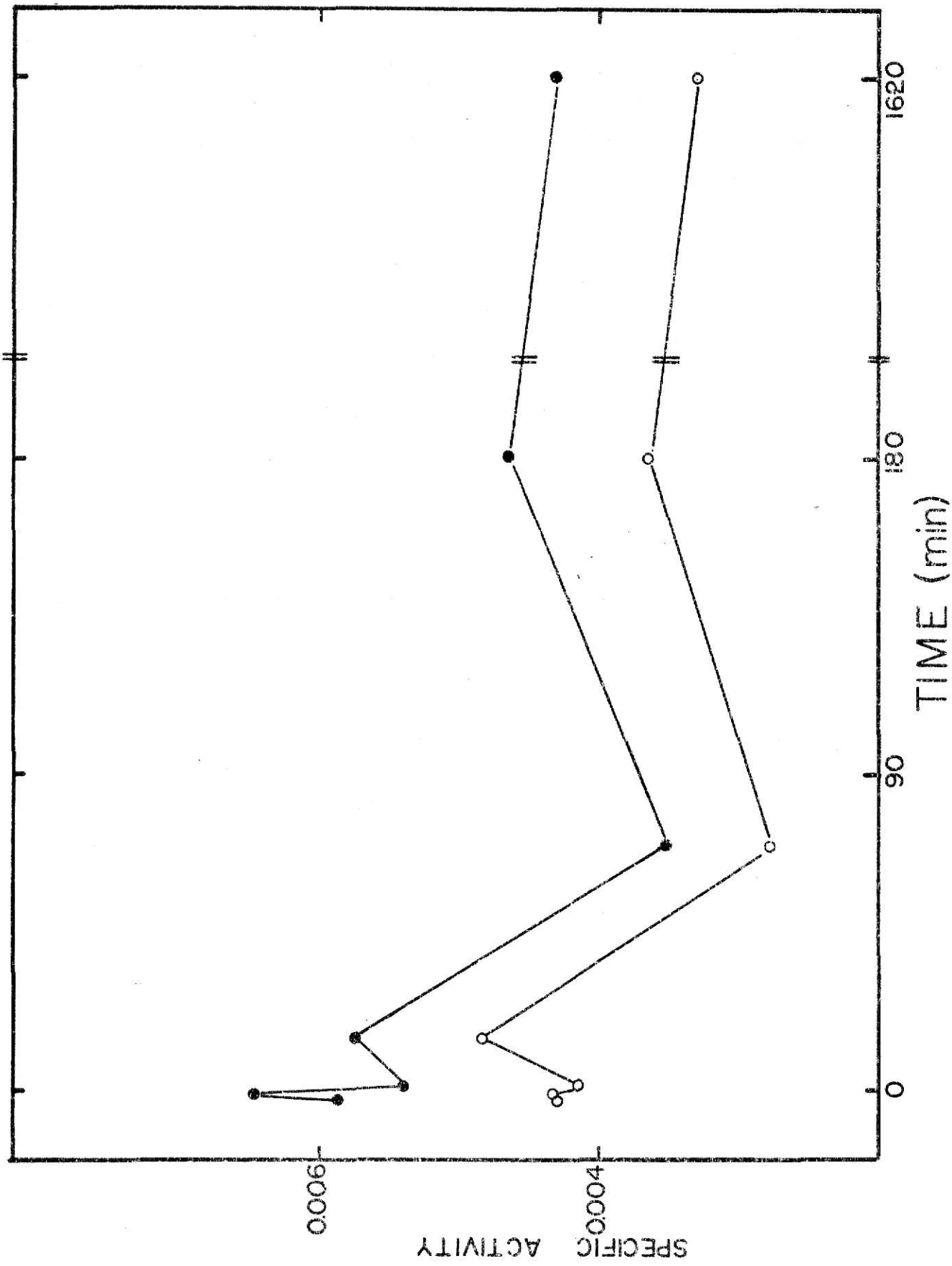
Because of the stabilizing effect of magnesium on ATP, the energy source of the aminoacylation reaction, a possible effect of low Mg^{++} concentration on the VRS activity mediated by a drop in the ATP concentration was tested. In order to increase the permeability of the punctured cell wall, E. coli strain NP4 was infected with bacteriophage T_4 and samples were removed at 0, 1, 2, 3, 4, and 5 minutes after infection, mixed with chloramphenicol to a final concentration of 100 µg/ml and further incubated until 15 minutes after infection. An enzyme assay using half the normal ATP concentration did not reveal any significant difference in activity for the 0, 1, and 5 minute extract. (Data not shown)

Based on these in vitro results we assumed that fluctuations in the magnesium ion concentration, due to permeability changes of the host membrane after T_4 infection, do not explain the necessity of a τ synthesis under the normal laboratory conditions employed. The following experiments were designed to reveal an advantageous property of the modified enzyme under conditions, where the normal steps in phage production are prolonged.

NP4 was grown on Benzer Broth at 37°C, treated with chloramphenicol and infected with bacteriophage T_4. Before the treatment with the antibiotic as well as before and after phage addition aliquots were removed and assayed for VRS and ARS (arginyl-tRNA synthetase) activity. Figure 12 shows that due to phage infection under conditions where protein synthesis was arrested, the specific activity of these enzymes declined to nearly 50% after 70 minutes.

Figure 12. Infection with T_4 in the presence of chloramphenicol.

E. coli strain NP4 was grown at 37°C, and after removal of a 10 ml sample the rest of the culture received chloramphenicol. Further samples were withdrawn after infection with bacteriophage T_4. The specific activity of VRS ● — ● and ARS o — o was determined.


SPECIFIC ACTIVITY
0.006
0.004
0
90
180
1620
TIME (min)

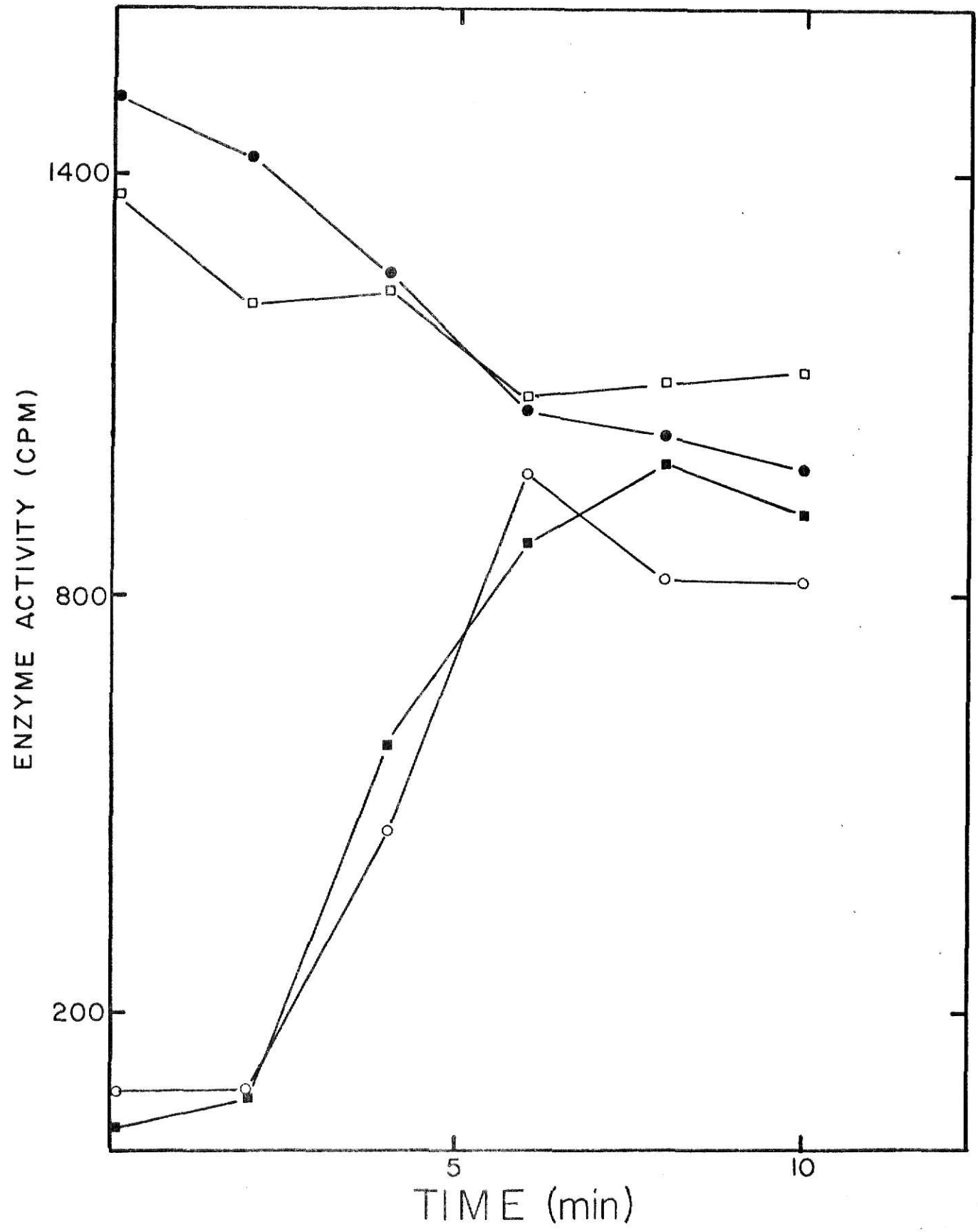
The slight increase after 3 hours is probably due to a loss in smaller proteins that can penetrate the defective cell wall. This effect, however, is not specific for valyl-tRNA synthetase and can therefore not be interpreted as a possible reason for modification.

More specific for VRS are the findings of enhanced stability of the modified enzyme when stored at 4°C for several weeks. Extracts from T_4 infected E. coli cells were prepared and assayed for synthetase activity as well as urea stability of the host VRS. After storage for approximately 3 weeks at 4°C these extracts were reassayed under the same experimental conditions. Figure 13 shows that the activity of the sample removed at 0 minutes after infection dropped from 1500 CPM to less than 100 CPM over this period of time, while increased activity is preserved in those samples, where modification of the host enzyme has proceeded. The curve of urea stable enzyme, which was obtained directly after preparation of these extracts, appeared to be nearly identical to the curve, which represented the residual activity after 17 days storage. The stability of the arginyl-tRNA synthetase after this time demonstrated that this effect was specific for valyl-tRNA synthetase.

This so far unknown feature of modification adds to the general conclusion that r confers an increased stability upon the host enzyme. The physiological significance, however, remains elusive.

Figure 13. Time effects on the stability of host and phage specific VRS.

E. coli strain NP4 was grown on casamino acid medium at 37°C, infected with bacteriophage T_4 and assayed at various times after infection for VRS activity ● — ● and urea stable VRS activity ■ — ■. After storage for 19 days at 4°C these extracts were reassayed under the same experimental conditions, VRS activity ○ — ○, ARS activity □ — □.


1400
800
200
ENZYME ACTIVITY (CPM)
5
10
TIME (min)

DISCUSSION

The viral modification of valyl-tRNA synthetase (VRS) in E. coli has been investigated in several laboratories. Bacteriophage T_4 induces a temperature stable VRS activity in E. coli mutant strain NP29 (38), which contains a thermolabile host enzyme, a high molecular weight form of VRS in strain NP2 (10), and a urea stable VRS activity in strains NP2 and NP4 (29, 39). This apparent diversity in phage effects seemed to be accompanied by differences in their temporal appearance (34, 38), depending upon the property that was assayed. Our work with E. coli strains NP4, NP2, and NP29 was concerned with the diversity of these reports and the results allow the following conclusions.

The phage induced conversion of host enzyme to a urea stable form on the one hand and a high molecular weight molecule on the other appears as one reaction that confers both features upon the enzyme simultaneously.

Protein synthesis is required for this reaction to occur. Since the phage specified factor τ is thought to be responsible for urea stability, the synthesis of τ appears to be a necessary step for the expression of both characteristics.

The urea stable VRS form appears in T_4 phage infected E. coli strains NP2, NP29, and NP4 on the same schedule after infection.

VRS modification is initiated 2 minutes after infection and completed between the sixth and seventh minute of infection when cells are grown on casamino acid medium at 30°C.

A detailed analysis of the kinetics of modification was carried out with regard to the onset and the velocity of this reaction. Under the conditions we employed, the growth of strain NP4 was limited by the temperature. In the

range from 20° to 37°C the overall velocity of cell division increased 2.5 fold upon a temperature raise of 10°C (Q_{10} = 2.5). Analogous to the complexity of cell growth, the modification reaction represents a complex of at least 3 different reactions; vs gene transcription, translation of τ messenger and τ*VRS complex formation. It is not surprising to find then that the overall velocity of these processes reveal approximately the same temperature dependence characteristic for the growth rate. Between 20 and 30°C the Arrhenius plot for the velocity of modification at different temperatures yields two distinct parallel lines, one representing the Benzer Broth culture, the other one the M9 culture (Figure 6b). This indicates that the energy source is responsible for different magnitudes in velocity at a constant temperature, but does not influence the change in velocity in response to an increase in temperature, which appears then to be the only rate limiting factor of this complex reaction. Above 30°C that situation appears to be different. While modification of VRS in the M9 culture is still determined by a Q_{10} of 2.7, this value is somewhat reduced in the Benzer Broth culture when shifted to 37°C. The smaller increase in reaction velocity of 1.9 fold reflects the diminished influence of temperature under these conditions, indicating that other factors now become rate limiting. Since the growth rate does not express this unique behavior under the same conditions, it is rather unlikely that these rate limiting influences concern the process of transcription and translation. It is more probable, therefore, that the reactions involving the attachment of τ to the host VRS are responsible for the decrease in Q_{10}. Although little information about the τ*VRS complex formation is available, the reaction can be at least partially described as a nonenzymatic process of the type: $n\tau + n\text{VRS} \rightleftharpoons \tau_n{}^*\text{VRS}_n$.

Because an *in vitro* modification with just these components has been demonstrated (29), it is reasonable to exclude the necessity of an enzymatic activity, lowering the activation energy for the τ attachment. With this in mind the relative high temperature dependence of VRS modification becomes much clearer. A Q_{10} of 2.7 speaks much for an uncatalyzed reaction, while a Q_{10} of 1.9 indicates the presence of an enzymatic activity. Since the overall velocity of a chain reaction is determined by its slowest step and transcription and translation are known to be enzyme catalyzed, the attachment of τ factor to the host VRS might cause the high temperature dependence of the modification reaction. However, this is not in agreement with the Q_{10} of 1.9 found for the Benzer Broth culture between 30 and 37°C. It might be possible, therefore, that the attachment step actually is subject to an increase in velocity corresponding to a Q_{10} of 2.7. However, the rate constant for this uncatalyzed reaction might be higher now than the rate constant of transcription and translation, which respond only slightly to increases in temperature, because of enzyme catalysis (Q_{10} = 1-2). The rate limiting step in this case might then be the inability of the enzymatic synthesis of τ to keep up with its attachment to the host enzyme at these higher temperatures.

While the velocity of modification was shown to be strongly dependent upon the growth media and the temperature in regard to its magnitude, its onset appears to be controlled differently. Several steps in the lytic growth of bacteriophage T_4 have to occur before urea stable VRS can be detected. Adsorption of phage, injection of DNA, transcription, translation and τ*VRS complex formation are the essential reactions so far known. It has to be pointed out, however, that this list is probably not complete, and

several intermediary steps might be necessary before an alteration of the enzymic property of the enzyme in question can be detected. Nevertheless, the end product of this reaction is the peptide $\underline{I}$, and the time at which the first modified VRS molecule can be detected is necessarily in direct relationship to the overall velocity of these processes. With the proper Arrhenius plot, the influence of temperature and media were determined and allowed the conclusion that over the temperature range of 10 to 25°C the modification reaction is initiated completely independently of the energy source. That is somewhat surprising since modification is mediated by several reactions (transcription and translation), which are essential for growth and continuous modification of VRS, both of which were shown to be influenced by the carbon source. In addition the Q_{10} for initiation below 25°C is unusually high compared to other biochemical reactions in the cell. An increase in temperature must therefore especially effect those types of reactions that are specifically involved in the onset of modification but do not play a role for normal growth. Two steps in the reaction chain are of special interest in this regard. Several workers have reported that the adsorption of phage to the cell surface is strongly dependent on the temperature. Stent proposed a two step mechanism for this process in which the second step is much more sensitive to temperature changes than the collision frequency. This, he argued is the reason why the adsorption rate is 10 fold higher at 25°C than at 5°C (50). Thus it is possible that the first reaction of the sequence, the adsorption of phage, is so temperature dependent that it becomes rate limiting for the whole reaction chain. If one assumes that the rate constant K_1 of at least one of the reactions preceding transcription is significantly smaller than subsequent rate constants (K_2 to K_n), and furthermore takes into

consideration that the media is similar in pH and ionic composition and therefore does not play a role in phage adsorption (50), the apparent unimportance of media for the onset of modification appears logical. Since the differences in growth rate due to various energy sources were approximately 1.8 fold at 10°C and 1.5 fold at 30°C, the velocity of transcription and translation could not differ more in these cultures than determined by these rates. Assuming that $K_1 << K_{2,3...n}$, the overall velocity will be mainly determined by K_1, and small changes in K_2, K_3, etc. due to different energy sources will escape experimental detection. From the data given by Stent (50) the Q_{10} for the adsorption of phages calculates as approximately 3.2. As indicated before the last step of the chain reaction, the attachment of r to VRS is also an uncatalyzed reaction with a Q_{10} of 2.7. Thus, the overall reaction including all steps from the adsorption of phage to the final modification of host VRS appears to be determined in its velocity by two uncatalyzed reactions, resulting in a Q_{10} of 4.7. The drop to a Q_{10} value of 2.2 above 25°C indicates that the overall reaction velocity is now determined by an enzymatic action rather than the two nonenzymatic steps. Due to their 5 fold increase the nonenzymatic rate constants exceed the enzymatic reaction rates in this temperature range, in that the latter reactions become rate limiting for the overall reaction velocity.

In several experiments we attempted to elucidate the regulation of the T_4 specific vs gene. From experiments carried out at 25 to 37°C it was rather obvious that VRS modification appeared as an early function of the bacteriophage. However, the report of several workers that the early genes of T_4 were transcribed sequentially made a more detailed analysis of the vs gene expression desirable. Speculating on Hosoda's and Levinthal's results

(22), we were able to define the appearance of urea stable VRS activity. When cells were infected at 25°C urea stable VRS activity appeared first after approximately 4 minutes and reached its maximum value at the twelfth minute of infection. Thus, in accord with Hosoda and Levinthal, urea stability must be either a rather late member of the class A proteins (0-10 minutes), or a very rapidly appearing class B protein (0-20 minutes). In order to support these theoretical considerations, urea stability was compared with other early enzymes. Warner and Lewis (56) reported that the increase in dihydrofolic acid reductase activity occurring in T_4-infected cells was not subject to the same regulation as other phage induced early enzymes, like dCMP deaminase or deoxynucleotide kinase, and that dihydrofolate reductase was synthesized faster than these other enzymes. Our findings (Figure 7) were in complete agreement with their results in that FH_2 reductase preceded the expression of the deaminase activity. VRS modification could be detected somewhat earlier than the latter enzyme, but resembled closely the kinetic pattern of its synthesis. This speaks for a difference in regulation of the dihydrofolate reductase gene and the vs gene, with the indication of FH_2 reductase being a class A protein and urea stability a class B member. The deoxynucleotide kinase could be referred to as a class C enzyme. It must be pointed out, however, that this classification of the vs gene product(s) is based upon the temporal appearance of VRS urea stability, which does not necessarily reflect the kinetics of τ synthesis. As already indicated, the attachment of τ to the VRS is probably a nonenzymatic reaction and therefore compared to the action of FH_2 reductase a time consuming process (below 30°C). In addition, a threshold quantity for τ might exist that prolongs the time between translation of the vs gene

transcript and VRS modification, while the first few reductase molecules can be detected easily. Another reason for a possible difference in the sequence of transcription and temporal appearance of early proteins might be the difference in the length of polycistronic messages. If one assumes that the vs gene is transcribed at the end of a longer message and the dCMP deaminase gene at the beginning of a polycistronic message, the simultaneous appearance of dCMP deaminase activity and modified VRS might conceal that the corresponding gene transcripts belong to different classes of mRNA. Therefore it was necessary to classify proteins by the kinetic order of their transcription rather than their translation. We followed the procedure of Buchanan and his coworkers using different inhibitors to separate the infectious cycle into a period of transcription and translation. When transcription was blocked with rifampicin and translation inhibited by 5-methyl-tryptophan we obtained the same result reported by Lembach and Buchanan (26). The dihydrofolate reductase gene appeared to be transcribed in the presence of the tryptophan derivative, while dTMP kinase could not be detected under the same experimental conditions. Based on these findings Lembach et al. (26) defined the first enzyme to be an immediate early phage function and the latter a delayed early one. In our experiment the emergence of urea stability resembled closely the behavior of the dihydrofolate reductase, and one minute after restoration of protein synthesis approximately 44% of the VRS appeared to be modified; further modification occurred during the following 14 minutes of incubation. This strongly suggests that the vs gene is transcribed in the presence of 5-methyl-tryptophan, a property which could not be demonstrated for the deoxinucleotide kinase gene. Therefore these genes must be located on different operons with different regulatory

properties. Furthermore some translation of the $\underline{\tau}$-messenger must occur to account for the high level of VRS modification after one minute of protein synthesis. This leaves two possibilities: The $\underline{\tau}$ molecules synthesized in the presence of the tryptophan derivative are fully active, but only 44% of the normal amount is produced during this incubation period. In this case the $\underline{\tau}$ peptide might not contain any tryptophan moieties in its primary structure, or if so they are not involved in the binding to VRS. However, Boyd's unpublished results (8) concerning an abnormal acting modified VRS synthesized in the presence of 5-methyl-tryptophan and other amino acid analogues make this possibility unlikely. It is more probable, therefore, that the 44% modification reflects the synthesis of an abnormal $\underline{\tau}^*$, incapable of complete VRS modification, due to 5-methyl-tryptophan residues in the primary structure. The production of normal $\underline{\tau}$ molecules after restoration of protein synthesis promotes an exchange reaction between abnormal and normal $\underline{\tau}$ molecules manifested by a 20% increase of modification. Consequently the reaction τ + VRS ~ $\tau^* \rightleftharpoons \tau^*$ + VRS - τ can be concluded. Since this reaction is most probably uncatalyzed, as pointed out before, the increase of 20% in modification after addition of tryptophan might not reflect the magnitude of $\underline{vs}$ gene transcription in the presence of rifampicin. In an attempt to avoid this problem chloramphenicol was used as an inhibitor of protein synthesis. The effectiveness of this drug was already demonstrated, in that it blocks the modification of host enzyme completely. Consequently in the following experiment the urea stability of host VRS was found to be at the background level (approximately 3% in both experiments), when extracts were prepared one minute after restoration of protein synthesis. However, a better yield in modification than achieved in the 5-methyl-tryptophan experiment could not be

obtained. This was thought to be due to extensive mRNA degradation during the time period necessary for diluting out the chloramphenicol. When this time was shortened by 8 minutes in a second experiment the modification could be increased by approximately 10% and reached half the value of the control culture. More important was the striking similarity between the dihydrofolate reductase activity and VRS modification in these experiments. Although both proteins were synthesized in smaller amounts with use of both inhibitors as compared to the control cultures, the appearance of their activity definitely indicated their transcription in the presence of chloramphenicol. The level of deoxynucleotide kinase was found not to be increased under these conditions, while the dCMP deaminase behaved somewhat intermediately. These results strongly suggest that the transcription of dihydrofolate reductase and VRS modifying factor τ are subject to the same or very similar regulatory mechanisms, which are strikingly different from those of the dCMP deaminase and deoxynucleotide kinase gene. The mRNA for both genes accumulates in the absence of concomitant protein synthesis, from which we conclude that τ is an immediate early function of bacteriophage T_4.

In regard to their location on the linkage map of bacteriophage T_4 the similarity in regulatory properties of the vs gene and the dihydrofolate reductase gene is somewhat surprising. Since they are nearly half the length of the chromosome apart it has to be concluded that they are not part of the same transcriptional unit. In 1966 Bautz et al. showed that the e gene of T_4 is transcribed during the first two minutes of infection, followed by a period of greatly reduced transcription. Later in infection the rate of e specific mRNA was found to increase again (4). While the early produced e gene mRNA could not program the synthesis of active lysozyme in vitro, the

late transcript could be translated into an active enzyme (9). In addition it was demonstrated that contrary to most of the late genes the e gene is transcribed from the l strand of the T_4 chromosome (19, 23). These data might be applied to our system in the following way: As mentioned before the vs gene is located between the e and the rI gene of the phage genome. Thus it is quite possible that one of the promoters that can be recognized by the unmodified host RNA polymerase is located on the right of the e gene and immediate early transcription proceeds in the leftward direction. Therefore the e and the vs cistron and any sequences between them might be transcribed as one polycistronic message. The next well defined gene to the left of rI is gt, coding for α-glucosyl-transferase (53). Between these two markers three other genes were found (53), gene 55, known to be indispensible for the onset of late proteins (9), gene 49, involved in head completion (53) and gene nrdC, coding for a phage induced thioredoxin (52, 59). The fact that α-glucosyl transferase was shown to be delayed early (35) and Stevens hypothesis that the gene 55 product is host polymerase modifying peptide I poses the possibility that the immediate early transcript starting on the right side of e includes at least gene e, vs, rI, nrdC, 49 and 55, and is terminated between gene 55 and gt. The translation of this polycistronic message might not include the e product (9), but the following sequences. Gene 55 product might then combine to the core polymerase, thereby allowing the transcription of the late regions. Extensive RNA studies, however, are necessary to establish this hypothesis.

All the immediate early proteins found in T_4 so far represent essential functions for lytic growth. Their importance for the phage is expressed by the fact that special regulatory mechanisms were developed to allow a

selective transcription of just these genes by the host RNA polymerase. The existance of T_4vs^- mutants that do not confer urea stability upon the host VRS but grow with normal burst sizes, and my finding that conversion of VRS to a urea stable phage specific form occurs as an immediate early function apparently contradicts this generalization. Since it was not established that these mutants really lack a fully functional vs-product, the question whether the physiological role of this product justifies its immediate early transcription remains unsolved. So far our attempts to elucidate the physiological significance of modification have been unsuccessful in that we could not demonstrate an advantageous feature of modified enzyme under conditions (low Mg^{++} ion concentration or prolonged growth), which match special in vivo situations. On the other hand several favourable properties could be demonstrated in vitro. Neidhardt et al. reported a greater resistance of the phage enzyme to denaturation by heat or urea (39). We found that modified VRS was quite stable when stored at 4°C over a longer period of time, while the host form did not survive this treatment. From this we conclude that T_4 specific modification of E. coli VRS confers an enhanced stability upon the enzyme over a wide temperature and time range. With our lack of knowledge about the ecology of T_4 phages it is rather difficult to speculate on the physiological significance of genes, which appear to be nonessential under normal laboratory growth conditions. It is quite possible therefore, that this increased stability reflects a positive survival advantage under extreme in vivo situations, not examined in these experiments.

SUMMARY

The bacteriophage T_4 induced modification of valyl-tRNA synthetase was investigated in Escherichia coli wild type strains NP2 (K-12), NP4 (B) and a temperature sensitive mutant NP29 (derived from NP2). Modification began 2 minutes after T_4 infection and was completed after 7 minutes of infection in all three strains when grown in casamino acid medium at 30°C. Conversion of the host enzyme to a urea stable phage form occurred simultaneously with its conversion to a high molecular weight form and was dependent on protein synthesis.

A kinetic analysis of the onset and velocity of modification revealed that onset was temperature dependent but independent of the carbon source used for growth, while the velocity of modification was dependent both on temperature and carbon source.

A comparison of the modification of valyl-tRNA synthetase with other T_4 phage induced early enzymes suggested that urea stability occurs as a pre-early function of bacteriophage T_4. Sequential use of inhibitors of protein and RNA synthesis demonstrated that the mRNA for modification is transcribed in the absence of protein synthesis. Preliminary attempts to elucidate the function of the T_4 induced peptide $\underline{r}$, which is thought to be in part responsible for modification, were inconclusive but led to the finding that urea stable enzyme appears to be more stable over a longer period of time than unmodified host valyl-tRNA synthetase.

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BACTERIOPHAGE T_4 INDUCED MODIFICATION OF VALYL-tRNA SYNTHETASE IN *ESCHERICHIA COLI*. AN ANALYSIS OF THE KINETICS AND REGULATION.

by

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Bacteriophage T_4 is known to induce the formation of a urea stable valyl-tRNA synthetase in Escherichia coli wildtype strain NP2 (K-12), a high molecular weight form of the enzyme in strain NP4 (B-strain), and an in vitro stable form of the enzyme in the temperature-sensitive mutant NP29 (derived from NP2). The phage vs gene product, a small peptide called τ, is thought to be in part responsible for these alterations of the host enzyme. The object of my work was to investigate the kinetics of modification in these three E. coli strains and to examine the regulation of the phage specific peptide τ.

It was found that modification as judged by urea stability tests, sucrose density gradient centrifugation or in vitro enzyme activity respectively occurred in E. coli strain NP2, NP4 and NP29 on the same schedule. Modification appeared to be initiated after 2 minutes of T_4 phage infection and was completed after 7 minutes of infection, when cells were grown in casamino acid medium at 30°C. Using chloramphenicol as an inhibitor of protein synthesis modification was shown to be dependent on protein synthesis. Conversion of host valyl-tRNA synthetase to a urea stable form was found to occur simultaneously with its conversion to a high molecular weight molecule.

Growth and infection of E. coli strain NP4 on different media and at different temperatures gave information about the kinetics of modification. Initiation of modification was found to be dependent on the temperature but independent of the carbon source used for growth, while the velocity of the modification reaction was dependent both on temperature and growth media.

The regulation of the T_4 specific *vs* gene product in infected *E. coli* cells was investigated by comparison of appearance of urea stable synthetase activity with other phage specific early enzyme activities. Sequential use of inhibitors of protein and RNA synthesis demonstrated that the mRNA containing the information for modification was transcribed in the absence of concomitant protein synthesis. Based on these experiments it was concluded that modification of valyl-tRNA synthetase in *E. coli* occurs as an immediate early function of bacteriophage T_4.

Urea stable enzyme was found to be more stable over a longer period of time than unmodified host valyl-tRNA synthetase. Other attempts to elucidate the function of modification and thus of the phage specific peptide *τ* were inconclusive and a final judgement in this regard has to await further evidence. However, these premisses allow the following hypothesis for T_4 phage induced modification of valyl-tRNA synthetase in *Escherichia coli*: One or two minutes after T_4 phage infection a phage *vs* gene is transcribed by host RNA polymerase. Translation of this mRNA produces a peptide *τ* (MW 10,000) which specifically interacts with valyl-tRNA synthetase (MW 100,000). This interaction confers urea stability upon the host enzyme and a conversion to a molecule complex with a molecular weight of 170,000, most probably through specific binding of a tRNA species. Though this new physical state of valyl-tRNA synthetase results in a more stable enzyme with reduced specific activity, the physiological advantage of modification remains unknown.