THE INDUCTION OF P1 PHAGE IN *E. COLI* THY$^-$ (P1)

A Thesis

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Robert Joseph Keating

August, 1972

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I should like to dedicate this thesis to my loving wife Bette, whose understanding and optimism were invaluable in all aspects of this investigation.

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ABSTRACT

This investigation has shown that analogs of thymine induced P1 phage from E. coli C thy-3284. The P1 phage was induced by the antibiotic mitomycin C and thymine starvation. The induction of P1 phage from strain C-328 by uracil derivatives indicates that direct interference with DNA synthesis is not necessary for P1 prophage induction.

UV irradiation, which caused phage induction and lysis in the lambda system, did not induce the P1 phage from C-328. This suggests a difference between the two phage systems. Inhibition of protein synthesis by chloroamphenicol did not induce P1 phage from C-328. An alteration in certain physical conditions, pH and osmotic pressure failed to induce P1 phage.

When E. coli C thy-3284 (P1) was incubated at 45°C, a bimodal growth response was observed. The significance of this bimodal response was associated with the presence of two phage types: a thermoinducible and non-thermoinducible P1 phage in the culture of C-328. It was shown that E. coli C thy-3284 (P1) was lysogenized by two phage types, such that some cells were lysogenized by the thermoinducible phage while others were lysogenized by the non-thermoinducible phage. It is suggested that one phage type was probably a mutant of the parent phage.

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INTRODUCTION

The temperate P1 phage lysogenizes various strains of E. coli. The P1 phage is a double stranded DNA phage (Thomas and McHattie, 1967). Ikeda and Tomizawa (1965) described the molecular weight of P1 phage to be 6×10^7 daltons. The P1 phage differs from other temperate prophages, such as lambda, in that the P1 prophage does not incorporate into the host chromosome during lysogeny (Boice and Luria, 1963). The P1 phage is an extrachromosomal replicating unit which exists in a circular form during the prophage state (Ikeda and Tomizawa, 1968). It appears that the P1 phage is immunologically different from other phages in that no serological crossreactions between it and other phages have been reported (Bertani, 1958).

Although lysogeny in bacteria was described in 1921 (Stent, 1963), lysogeny involving P1 phage was first described by Bertani in 1951. He isolated the P1 phage from Escherichia coli strain 'Li'. This P1 phage formed turbid plaques, 0.2 to 0.3 mm in diameter, when plated on Shigella dysenteriae (Bertani and Nice, 1954). The burst size of P1 phage when plated on various indicator strains of E. coli and S. dysenteriae varies between 150 to 350 (Bertani, 1958).

Several variants of P1 phage have been described. Bertani (1954) described P1v a virulent mutant of P1 that had lost its ability to

lysogenize E. coli and S. dysenteriae. Lennox (1955) isolated P1kc which forms larger plaques than the parent phage P1k on E. coli K-12. A defective prophage P1dl has been isolated from E. coli K-12 strains (Luria, et al., 1960).

Melechen and Skaar (1962) described P1b a mutant of P1kc, which was lysogenic for E. coli B3. Kondo and Mitsuhasi (1964) isolated P1CM a chloroamphenicol resistant mutant of P1kc, from E. coli K-12. Scott (1970a) isolated a derivative of P1CM, P1 cryptic, which is a defective phage. A thermoinducible variant of P1CM phage was reported by Rosner (1972).

Lennox (1955) studied the ability of P1 phage to transduce a large variety of genetic markers, including argenine, galactose and tryptophan, between strains of E. coli and S. dysenteriae. Adams and Luria (1958), using P1 phage, have described the co-transduction of closely linked genetic factors between strains of E. coli K-12. They observed that lysogenization does not necessarily accompany transduction. The lactose utilizing ability has been reported to be transduced by P1 phage among strains of E. coli K-12 and S. dysenteriae (Luria, et al., 1960). Kondo and Mitsuhasi (1964) demonstrated the transduction by P1 phage of the chloroamphenicol resistance factor between strains of E. coli K-12.

Many investigators have reported that the P1 prophage is not transferred during bacterial conjugation. Jacob and Wollman (1959)

reported that they were unable to transfer P1 phage from donor to recipient cells during bacterial conjugation. Certain defective P1 prophages have been exchanged between bacteria by conjugation. Boice and Lauria (1963) reported that they were able to transfer Pldl, a defective prophage, by mating F^-lac recipients with F^+ or Hfr donors of E. coli K-12. The defective PldCM has been transferred between bacteria during conjugation (Scott, 1970a).

Several chemicals have been described as inducing agents for the P1 phage. Melechen (1964) described the effects of 5-bromouracil on inducing the P1 phage from E. coli B3. Mitomycin C has been reported to induce P1 phage from E. coli by Seno and Melechen (1964). Melechen (1964) found that an analog of tryptophan, 5-methyltryptophan, would induce P1 phage from the lysogenic E. coli.

Melechen and Skaar (1962) have reported that thymine starvation of a thymine requiring P1 lysogen induced P1 phage. Scott (1970b) has studied the thermoinducibility of clear plaque mutants of Plkc in strains of E. coli K-12. A thermoinducible mutant of P1CM has been reported by Rosner (1972).

Theoretical Considerations

Preliminary observations showed that P1 phage was induced when E. coli C thy-3284 (P1) was grown in 5-bromouracil medium. When the BU-induced P1 phage was plated on E. coli C, the indicator organism, two types of plaque morphology were observed. The induction of P1 from E. coli with 5-bromouracil was reported by Melechen (1964). Thymine starvation (Melechen and Skaar, 1962) and Mitomycin C (Seno and Melechen, 1964) have induced P1 phage from lysogenic E. coli. These inducing agents are known to interfere with DNA synthesis.

The initial objective of this research was to determine if DNA inhibitors are unique in inducing P1 phage from E. coli C thy-3284. Experiments were designed to test the effect of thymine analogs and Mitomycin C on strain C-328. Conditions which interfere with DNA synthesis, UV irradiation, and thymine starvation will be investigated as possible inducers of the P1 phage. To determine if inhibitors of RNA and protein synthesis will induce the P1 phage, strain C-328 will be grown in the presence of a uracil analog and chloroamphenicol respectively. The effects of certain physical factors, such as altered pH, the effects of osmotic pressure and elevated temperature, will be studied using strain C-328. The experiments in this investigation will be designed to determine which chemical or physical agents induce P1 from strain C-328.

The observation of two plaque morphologies when BU-induced P1 phage was plated on indicator organism provides an approach to the question of generalized transduction by P1 phages. Hayes (1968) has stated that any bacterial gene can be transduced by P1 phage. The question arises as to whether there are several P1 phages in each single bacterium, each capable of transducing a specific marker, or are there several P1 types in a lysogenic culture, one type per bacterium, each capable of transducing a particular region from its host chromosome? The presence of more than one P1 phage types in a lysogenic culture has been reported by Bertani (1951). It has been suggested (Luria, et al, 1960; Ting, 1962) that all P1 phages contain one or more elements readily exchangeable for a segment of the bacterial chromosome. A secondary objective of this investigation will be to characterize the P1 phage induced from strain C-328 by 5-bromouracil, and if possible, separate the two plaque morphology types. Escherichia coli C thy-3284 (P1) will be tested to determine if it is lysogenized by one or both of the phages which produce different plaque morphologies.

MATERIALS AND METHODS

Bacterial Strains

Escherichia coli C thy-3284 (P1) and E. coli K-12 Met.$^-$ thy-3291 (λ) were the lysogenic strains used to test the phage inducing ability of certain chemical agents and physical factors. Escherichia coli C and E. coli C thy-321 were used as indicator strain and hosts for the propagation of P1 phage. All thymine auxotrophe required 2 ug/ml of thymine for optimal growth in a glucose-salt synthetic medium.

The following bacteria strains used to determine the host range of induced phage were maintained in our laboratory:

Escherichia coli Strains	Temperate phage
E. coli K-12 Thy,$^-$ his$^-$334	-
E. coli K-12 Ura,$^-$His,$^-$Pan$^-$	-
E. coli K-12 Try$^-$444	-
E. coli K-12 Thy,$^-$ his$^-$159	-
E. coli K-12 (434)	(434)
E. coli C 600 (21)	(21)
E. coli B	-
E. coli B/r	-

E. coli B Met$^-$	-
E. coli 15T$^-$	-
E. coli W 3104 (λ)	(λ)
E. coli 3704	-

Salmonella Typhimurium Strains	Temperate phage
S. Typhimurium 22 (P22)	P22
S. Typhimurium 19 Met$^-$	-

(- denotes the presence of a phage was unknown to the investigator.)

All bacteria strains were maintained on nutrient agar slants and stored at 4°C. Stock cultures were prepared monthly.

Chemicals

Thymine, histidine, methionine, uracil, pantothenic acid and Mitomycin C were purchased from the Nutritional Biochemical Corporation. 5-Aminouracil, 5-bromouracil, 5-iodouracil, 5-chlorouracil, 5-acetyluracil and 5-azauracil were obtained from Calbiochem. Chloroamphenicol was acquired from Sigma Biochemical Corporation. Other chemicals were of analytical grade and obtained from local chemical suppliers unless otherwise specified.

Glassware

All glassware used in the experiments were acid cleaned with an acid cleaning solution (0.5 gm sodium chlorate, 0.5 gm sodium nitrate in one liter of concentrated sulfuric acid). The glassware was rinsed thoroughly with distilled and deionized water and allowed to air dry. Acid cleaned glassware, including pipettes, were sterilized by autoclaving at 121°C of 15 psi of steam pressure. After sterilizing certain glassware was dried in a hot air oven at 160°C for 30-60 minutes.

Media and Solutions

a. MS-7 salt solution

1.0 gm	KH_2PO_4
0.5 gm	$Na_3C_6H_5O_7 \cdot 2H_2O$
0.7 gm	$MgSO_4 \cdot 7H_2O$
1.0 gm	NaCl
0.1 ml	$FeCl_3$ (0.5% aqueous solution)
2.0 gm	$(NH_4)_2HPO_4$
950.0 ml	H_2O

This solution was adjusted to pH 6.7 by adding 0.5 ml of 6 N HCl before autoclaving.

b. MS-7 medium

MS-7 salt solution	92.5 ml
Glucose (20% stock solution)	5.0 ml
Thymine (200 ug/ml stock solution)	0.25 ml

c. 5-Bromouracil-medium (BU-medium)

BU-medium was the same as MS-7 medium except that 5-Bromouracil (10 ug/ml) was added in place of thymine.

d. Limited-glucose medium

This medium was prepared by adding 0.04% glucose instead of 1% glucose as described for MS-7 medium.

e. L-broth and L-agar

10.0 gm	Tryptone
5.0 gm	Yeast-extract
5.0 gm	NaCl
1000.0 ml	H_2O (distilled-deionized)

This medium was adjusted to pH 7.0 with 1 N NaCH and sterilized by autoclaving. Two ml of 50% glucose and 2.5 ml of 1 M $CaCl_2$ were added to the medium.

L-agar was the same as L-broth except that 10 gm of agar was added to 1000 ml of medium. Soft agar contained 7 gm instead of 10 gm of agar per liter of medium.

To assure purity of bacteria strains (biochemical mutants and lysogens) marker checking was made during all experiments.

b. Turbidity measurements

To determine the effects of chemical agents and physical factors as inducing agents, the optical density of the culture was monitored at hourly intervals. A bacterial culture containing 10^7 cells/ml, was incubated in the presence of the appropriate chemical agent or physical factor. The optical density was measured with a colorimeter (Klett-Summerson), using a #42 blue filter. In certain instances a continuous decrease in the optical density of the culture was indicative of phage lysis.

c. Preparation of phage lysate

At the time when the optical density reading was made from an incubating bacteria culture 5 ml samples were collected. These samples were centrifuged at 5,000 rpm for 15 minutes to remove bacterial debris. Chloroform (0.5 ml) was added to each sample if not used immediately. They were stored at $4^{\circ}C$ until used. These samples are described elsewhere as the phage lysates.

d. Spot test for the presence of phage

A sterile inoculating loop was used to spot phage lysate on the indicator bacterium (or other bacteria) grown on L-agar plates. The

f. Peptone broth

9.0 gm	NaCl
3.0 gm	H_2O (distilled-deionized)

This medium was adjusted to pH 7.0 with 1 N NaOH.

All media and solutions were sterilized by autoclaving at 121°C or 15 psi pressure, for 15 minutes.

Phage Induction

a. Preparation of inoculum

Isolated colonies were selected from a plate culture and transferred to 5 ml of limited-glucose medium in a 19 x 55 mm test tube. The limited-glucose culture was incubated overnight at 37°C on a water bath shaker (American Optical Company). One ml of the overnight culture was inoculated into 99 ml of MS-7 medium in a 250 ml Erlenmeyer flask. The MS-7 medium culture was incubated for eight hours at 37°C on a rotary shaker.

The cells from the MS-7 medium were harvested on a sterile millipore membrane filter (Millipore Filter Corp.). The cells were washed twice with 50 ml of MS-7 salt solution. Cells were resuspended in 20 ml of MS-7 salt solution. This cell suspension was used to inoculate the appropriate quantity of media to yield the desired cell concentration for growth and phage liberation studies.

plate was incubated overnight at 37°C before observing plaque formation. Indicator strain inoculum was prepared by growing L-broth culture overnight.

e. Plaque assay

The methods used for the assay of phage in this investigation are those described by Adams (1959). Three ml of soft L-agar and 0.5 ml of indicator strain were added to test tubes in a hot water bath (Acme Laboratory Equipment Co.) at 49°C. One ml of phage, serially diluted in peptone broth, was transferred to the soft agar. After gentle mixing the phage and bacteria were allowed to stand for 5 or 10 minutes before overlaying a thin-layer L-agar plates. The plates, after allowing the soft agar to solidify were inverted and placed in the incubator at 37°C. After 16 hours of incubation the number of plaques observed on each plate were counted and multiplied by the appropriate dilution factor. The results were recorded in terms of plaque forming units (pfu) per ml.

One Step Growth Curve

a. Preparation of P1 antiserum

Two albino rabbits, averaging 12 kg in weight, were used in the preparation of P1 antisera. Prior to inoculation with P1 phage the animal was bled and the serum collected and assayed for P1 neutralizing activity.

The phage induced by 5-bromouracil was titered at 10^{10} pfu/ml and injected into the rabbit subcutaneously twice a week for three weeks. The animal was bled one week after the last injection of phage. The collected blood, after permitting to clot, was centrifuged at 2,000 rpm for ten minutes. The supernatant antiserum was removed and stored at 4°C in sterile screw cap test tubes until assayed for anti-P1 activity.

b. Assay of anti-P1 activity.

The anti-P1 activity was determined on rabbit serum diluted 1/10, 1/100 and 1/1000. One-tenth ml of a phage suspension (10^7 pfu/ml) was transferred to 0.9 ml of diluted antisera. To stop phage and antibody reaction (antigen-antibody) 0.1 ml of the P1-antisera was transferred to 9.9 ml of peptone broth at five minute intervals. One-tenth ml of each dilution was mixed with 3 ml of E. coli C soft agar suspension, before overlaying on thin L-agar plates. The plate cultures were incubated at 37°C overnight. The number of plaques per ml pfu/ml, was determined. Failure to form plaques was an indication of anti-P1 activity.

c. Burst size

To determine the burst size of the P1 phage a one step growth experiment was prepared. A culture of E. coli C was grown to a

concentration of 3.4×10^7 cells/ml. Phage suspension was diluted to a titer of 1.38×10^7 pfu/ml. P1 phage antisera (K=27) was diluted, one to ten, in peptone broth. One-tenth ml of phage was transferred to 0.9 ml of E. coli C suspension, 2.0×10^6 cells per ml and mixed and allowed to stand for five minutes. One-tenth ml of the phage-bacterium suspension was added to 0.8 ml of the diluted antisera, mixed and allowed to stand for five minutes. This suspension was diluted one to ten with L-broth.

Two growth tubes were prepared. The first growth tube contained 3.9 ml of L-broth. The second growth tube contained 9.9 ml of L-broth. Each tube received 0.1 ml of the phage-bacteria-antisera mixture. At two minute intervals 0.1 ml samples from the first and second growth tube were mixed with indicator strain in soft L-agar medium and poured onto L-agar plates. After 16 hours of incubation at 37°C the number of plaques per ml was determined for the first and second growth tubes.

RESULTS

Preliminary observations in our laboratory revealed that E. coli C thy-3284 (P1) was induced when grown in the presence of 5-bromouracil. Therefore it was of interest to determine the uniqueness of 5-bromouracil as an inducing agent for this pseudo-lysogenic organism.

Growth Response of Strain C-3284 (P1) in BU-medium

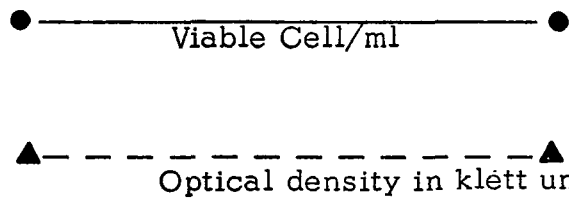
In order to evaluate the growth response of C-328 in BU-medium it is of value to know the growth response of C-328 under optimal culture conditions. Figure 1 illustrates the viable cells/ml and optical density of C-328 in MS-7 medium when plotted against time in hours. When a culture of C-328 was incubated at 37°C for seven hours there was a logarithmic increase in viable cells from 3.3×10^7 cells/ml to 1.9×10^9 cells/ml. The optical density curve paralleled that of the viable cells. No free phage were detected at two, four and seven hours using the phage spotting procedure previously described. The generation time for C-328 in MS-7 medium was 70 minutes.

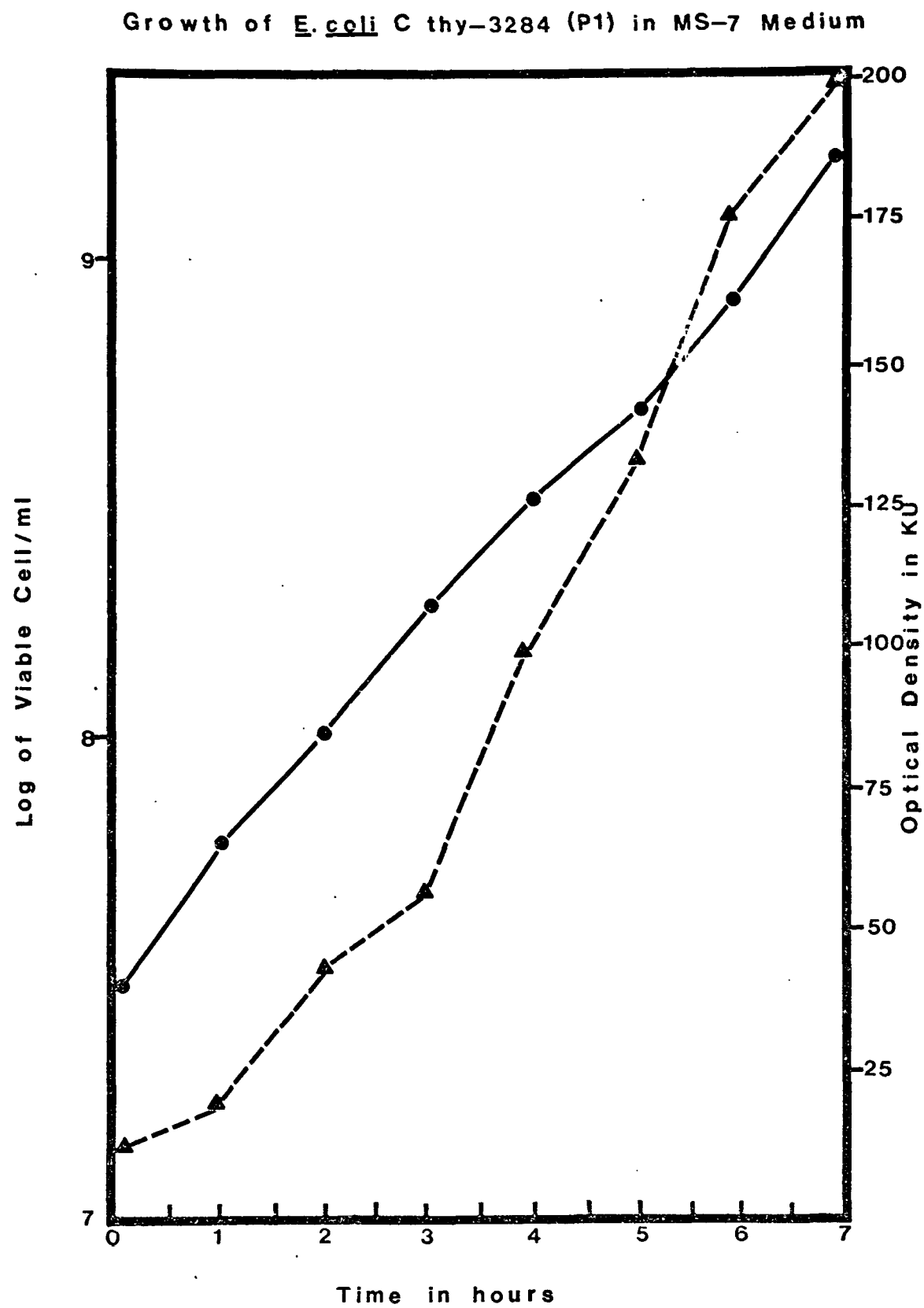
To characterize the growth response of C-328 in BU-medium, the viable cell count, optical density and phage titer were determined. Twenty ml of prepared inoculum was transferred to 80 ml of BU-medium to obtain 4.5×10^7 cells/ml. The culture was incubated at 37°C on a rotary

Figure 1

The viable cell number and optical density of

C-328 in MS-7 medium




Growth of E. coli C thy–3284 (P1) in MS–7 Medium
Log of Viable Cell/ml
9
8
7
Optical Density in KU
200
175
150
125
100
75
50
25
0
1
2
3
4
5
6
7
Time in hours

shaker and samples removed at thirty minutes interval for growth studies and phage liberation.

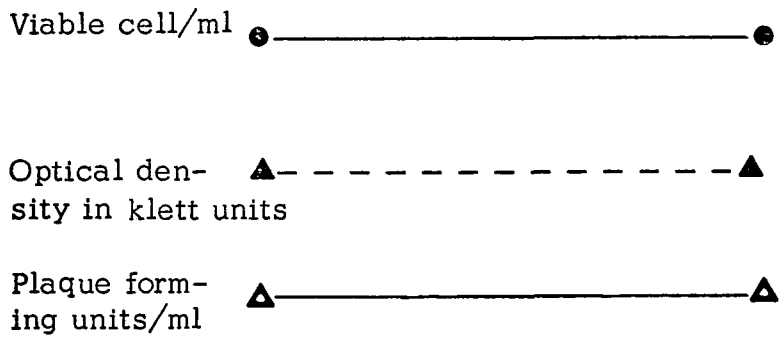
The results from growth studies of C-328 in BU-medium are illustrated in Figure 2. There was an initial increase in the viable cell count from 4.5×10^7 cells/ml to 1.67×10^8 cells/ml for the initial 150 minutes. This initial increase in viable cell number was followed by a continuous decline to 3.6×10^4 viable cells/ml at seven hours of incubation. A plot of the optical density readings paralleled that of the viable cell count except that the period of decline in optical density occurred after four hours of incubation rather than 150 minutes as described for the viable cell count.

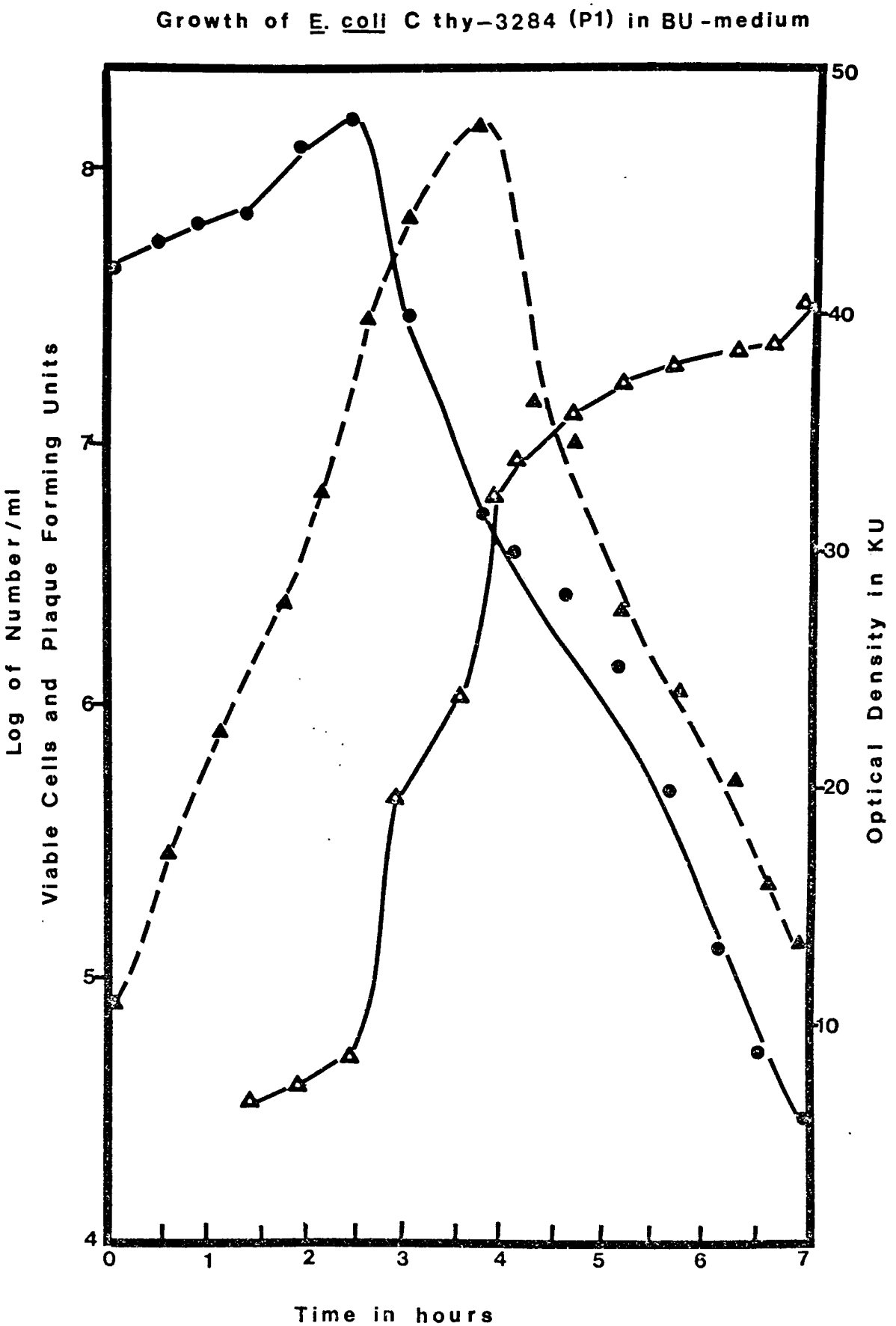
The decline in optical density was found to be caused by lysis of cells resulting in phage liberation. The delay in the decline in optical density after the decline in viable cells probably represents time required for phage propagation following prophage induction.

Phage liberation was detected by transferring a loopful of culture filtrate onto an indicator strain, E. coli C, and observing for plaque formation. Plaques were observed for samples taken after ninety minutes of incubation. The presence of phage in the culture prior to the decline in viable cell number or optical density at 240 minutes was indicative of certain cells in the non-synchronous culture being induced before others. The phage titer as illustrated in Figure 2 shows a logarithmic increase

Figure 2

Growth response and phage liberation of C-328 (P1) in BU-medium




Growth of E. coli C thy-3284 (P1) in BU-medium
Log of Number/ml
Viable Cells and Plaque Forming Units
Optical Density in KU
Time in hours
8
7
6
5
4
50
40
30
20
10
0
1
2
3
4
5
6
7

from 3.4×10^4 plaque forming units (pfu) per ml at 90 minutes to 3.3×10^7 pfu/ml at seven hours.

Characteristics of P1 Induction

To further characterize the induction of P1 phage from C-328 by 5-bromouracil the burst size, plaque morphology and host range were determined.

The K value for the antisera was determined to be 27. The burst size of 5-bromouracil induced phage was found to be 245 when grown on E. coli C. Figure 3 represents the number of plaque forming units per ml from the first and second growth tube when plotted against time in minutes.

When E. coli C was infected with P1 two plaque types were observed, after incubating for 18 hours, using a steriomicroscope (dissecting microscope). The larger plaque type was 1 mm in diameter with a smooth edge. The other plaque type was smaller, 0.3 mm, with a smooth edge.

The host range of P1 was determined by spotting phage lysate from 5-bromouracil induced phage on the various bacteria listed in Table I. Lambda phage and P1v, a virulent mutant of P1, were spotted on the same organisms. All strains of E. coli C were sensitive to BU-induced phage except E. coli C thy-3284, the host strain for P1 phage.

Figure 3

One step growth curve of P1 phage propagated on *E. coli* C

0 - 45 minutes plaques from first growth tube

45 - 70 minutes plaques from second growth tube

$$\text{Burst size} = \frac{\text{average of pfu second growth tube}}{\text{average of pfu first growth tube}}$$

$$245 = \frac{2.7 \times 10^{7} \text{ pfu/ml}}{1.1 \times 10^{5} \text{ pfu/ml}}$$

One Step Growth Curve of P1 Phage Propagated on E. coli C

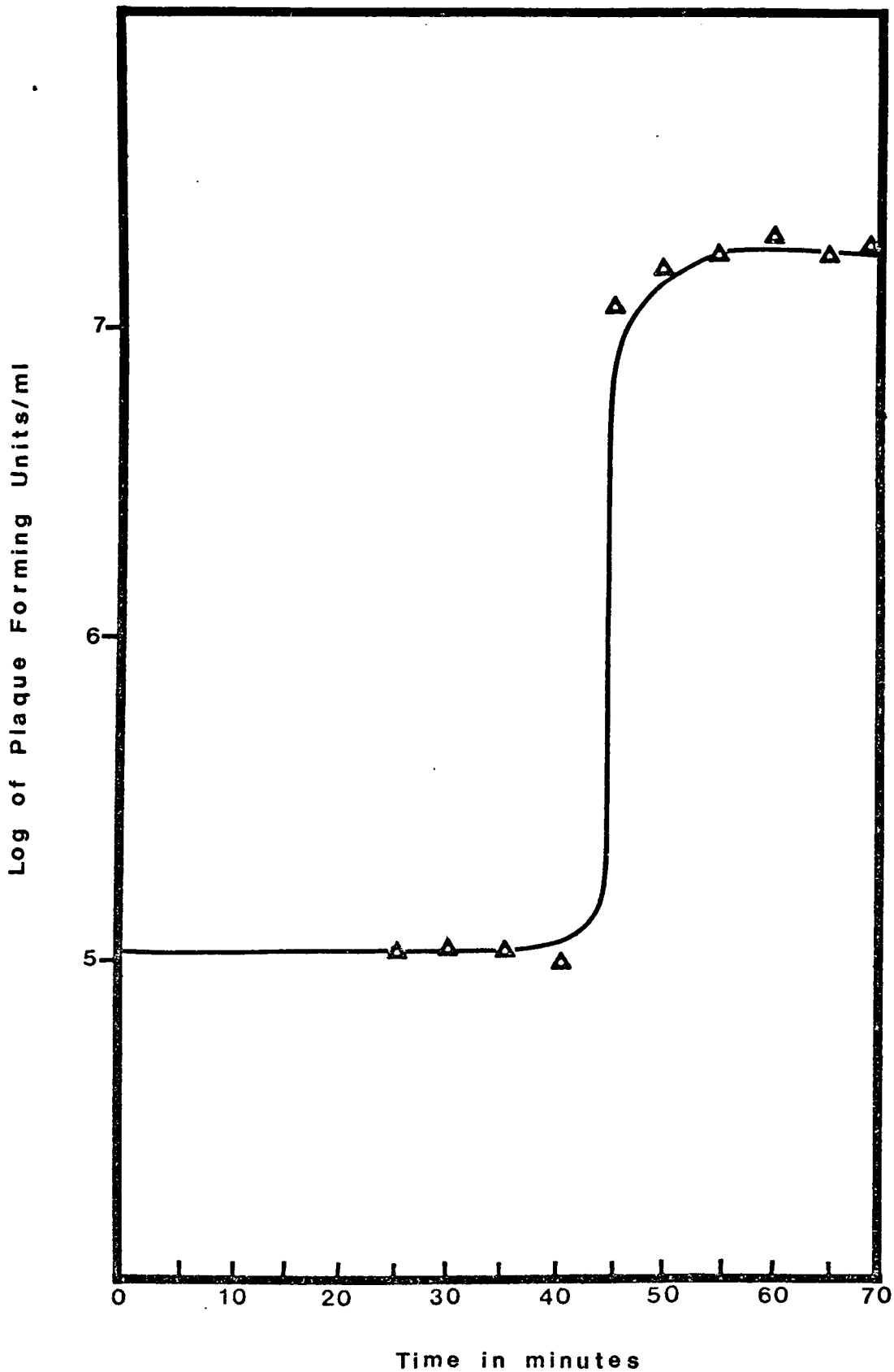


TABLE I

Bacterial strain	Sensitivity to phage P1	P1v	Lambda
E. coli C thy-3284 (P1)	-	+	+
E. coli C	+	+	+
E. coli C 600 (21)	+	+	+
E. coli C thy-321	+	+	+
E. coli K-12 (434)	-	+	+
E. coli K-12 Thy$^-$, his-334	+	+	+
E. coli K-12 Met$^-$, thy-3291 (λ)	+	+	-
E. coli K-12 Ura$^-$, Pan$^-$, His$^-$	+	+	+
E. coli K-12 Try$^-$ 444	+	+	+
E. coli K-12 Thy$^-$, his$^-$159	+	+	+
E. coli B	-	-	+
E. coli B Met$^-$	-	-	+
E. coli B/r	+	+	+
E. coli 15T$^-$	+	+	-
E. coli W3104 (λ)	+	+	-
E. coli 3704	+	+	+
Sal. typhimurium (P22)	-	-	-
Sal. typhimurium 19	-	-	-

The failure to lyse strain C-328 was due to this organism carrying the P1 prophage thereby being immune from superinfection with phage P1. All strains of E. coli K-12 were lysed by P1 phage except E. coli K-12 (434) which is lysogenic for the temperate phage 434. The strains of E. coli B were not sensitive to the P1 phage. A UV resistant mutant of E. coli B, E. coli B/r, was lysed by the phage. This sensitivity of E. coli B/r, may represent a change in cell wall surfaces, probably due to mutation when exposed to UV irradiation. The E. coli 15T$^-$, E. coli W 3104 (λ) and E. coli 3704 were sensitive to the P1 phage. The Salmonella strains did not lyse when challenged by P1 phage. The Salmonella strains' resistance may be due to improper receptor sites or immunity for P1 due to being lysogenized by a similar phage type.

All E. coli strains except the strains of E. coli B were lysed by P1v. The Salmonella strains were not sensitive to P1v. Lambda phage did not lyse E. coli 15T$^-$ or E. coli strains that carried a lambda prophage. The Salmonella strains did not lyse when spotted with lambda phage. The BU-induced P1 phage exhibits different host range projection when compared to the lambda phage and the virulent varient of the P1 phage.

Induction of P1 Phage with Other Agents

Another approach in investigating the uniqueness of 5-bromouracil as a phage inducing agent involved a study of the ability of certain chemical

and physical factors that may induce E. coli C thy-3284 (P1), (C-328). These chemical agents and physical factors included: Mitomycin C, UV irradiation and thymine starvation, chemical agents and conditions known to affect DNA synthesis; chloroamphenicol, a protein synthesis inhibitor; 5-aminouracil, 5-acetyluracil, 5-carboxylic acid uracil, 5-iodouracil, 5-chlorouracil and 5-azauracil derivatives of uracil; acid and alkaline pH, osmotic pressure and elevated temperature.

a. Mitomycin C

To determine if mitomycin C would induce C-328 1 ml (1 mg/ml) of this antibiotic was added to 99 ml of MS-7 medium and observed for a decline in optical density during incubation at 37°C. As shown in Figure 4 the optical density of the culture increased three-fold during the initial two hours of incubation. A continuous decline in the optical density was observed for the next four hours. When culture filtrate samples taken at 180 and 360 minutes were spotted on the indicator organism, lysis was observed. Mitomycin C did induce the P1 phage from strain C-328.

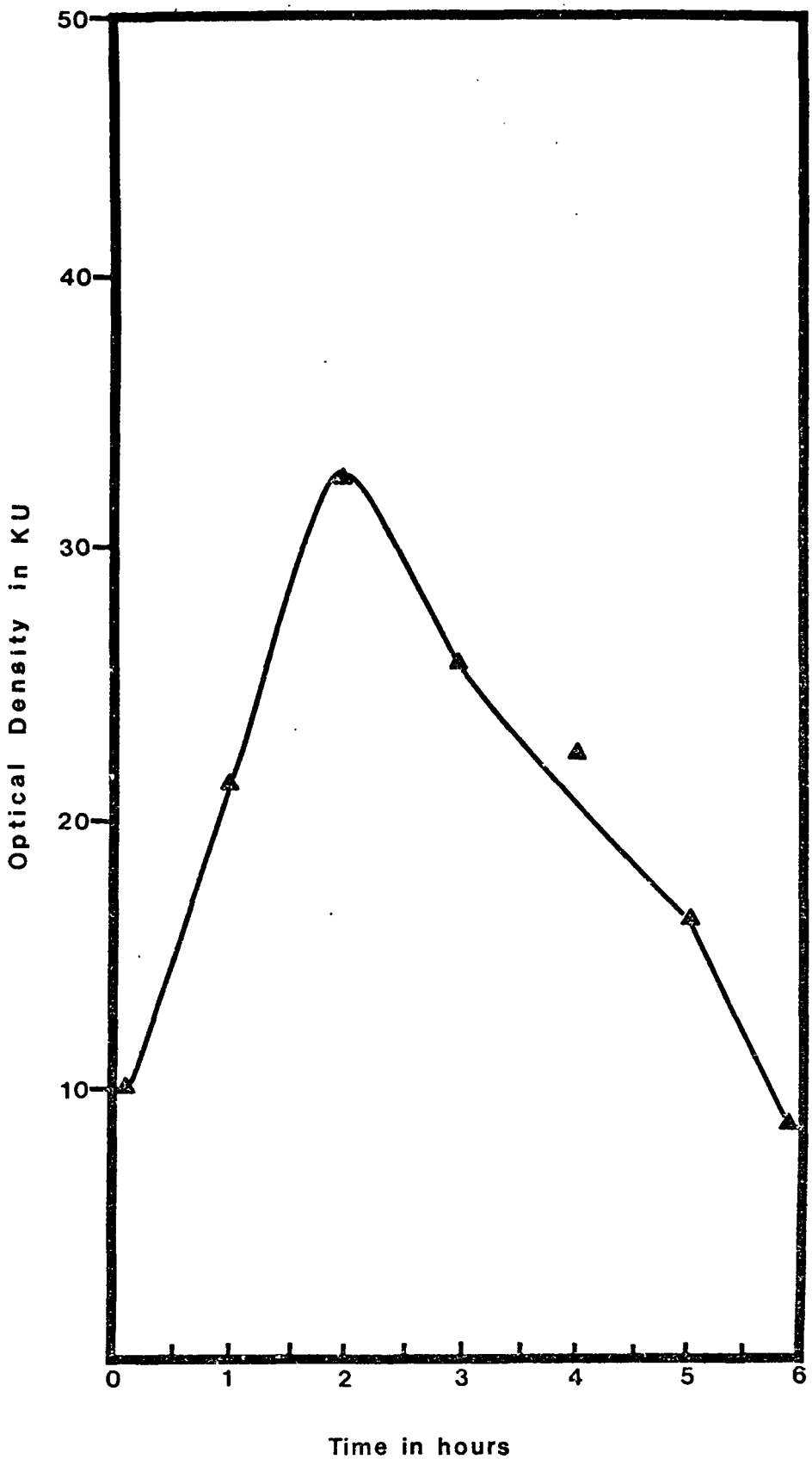
b. UV irradiation

To determine whether UV irradiation would induce P1 from strain C-328 20 ml of actively growing cells was transferred from MS-7 salt solution to sterile petri plates and exposed to UV light. The

Figure 4

The effect of Mitomycin C on strain C-328

The Effect of Mitomycin C on Strain C-328



cells were exposed to a UV germicidal lamp (G1528) for fifteen seconds. Immediately after irradiation the cells were inoculated into 80 ml of MS-7 medium.

The optical density readings of irradiated cultures of C-328 and E. coli K-12 Met$^-$, Thy$^-$ (λ) is presented in Figure 5. The optical density of the UV irradiated culture of C-328 increased during seven hours of incubation. After additional incubation of the culture for 18 hours there was no visible decrease in turbidity. No phage was detected when culture filtrate of 7 or 18 hours was spotted on indicator organism.

The lambda lysogen of E. coli K-12 when exposed to UV light under similar conditions as employed for strain C-328 did undergo lysis. Lambda phage lysis was detected by a decline in the optical density after 60 minutes and the presence of lambda phages in the culture filtrate at seven hours. The induction of phage from the lambda lysogen, E. coli K-12, indicated that the proper UV light exposure had been obtained.

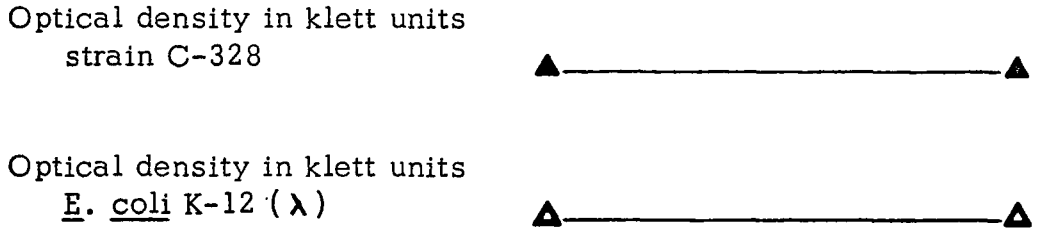
c. Thymine starvation

To examine the effect of thymine starvation on strain C-328 MS-7 medium free of thymine was inoculated with C-328 and incubated at 37°C for six hours. There was an initial increase in the optical density of the culture from 20 to 35 klett units for the initial 180

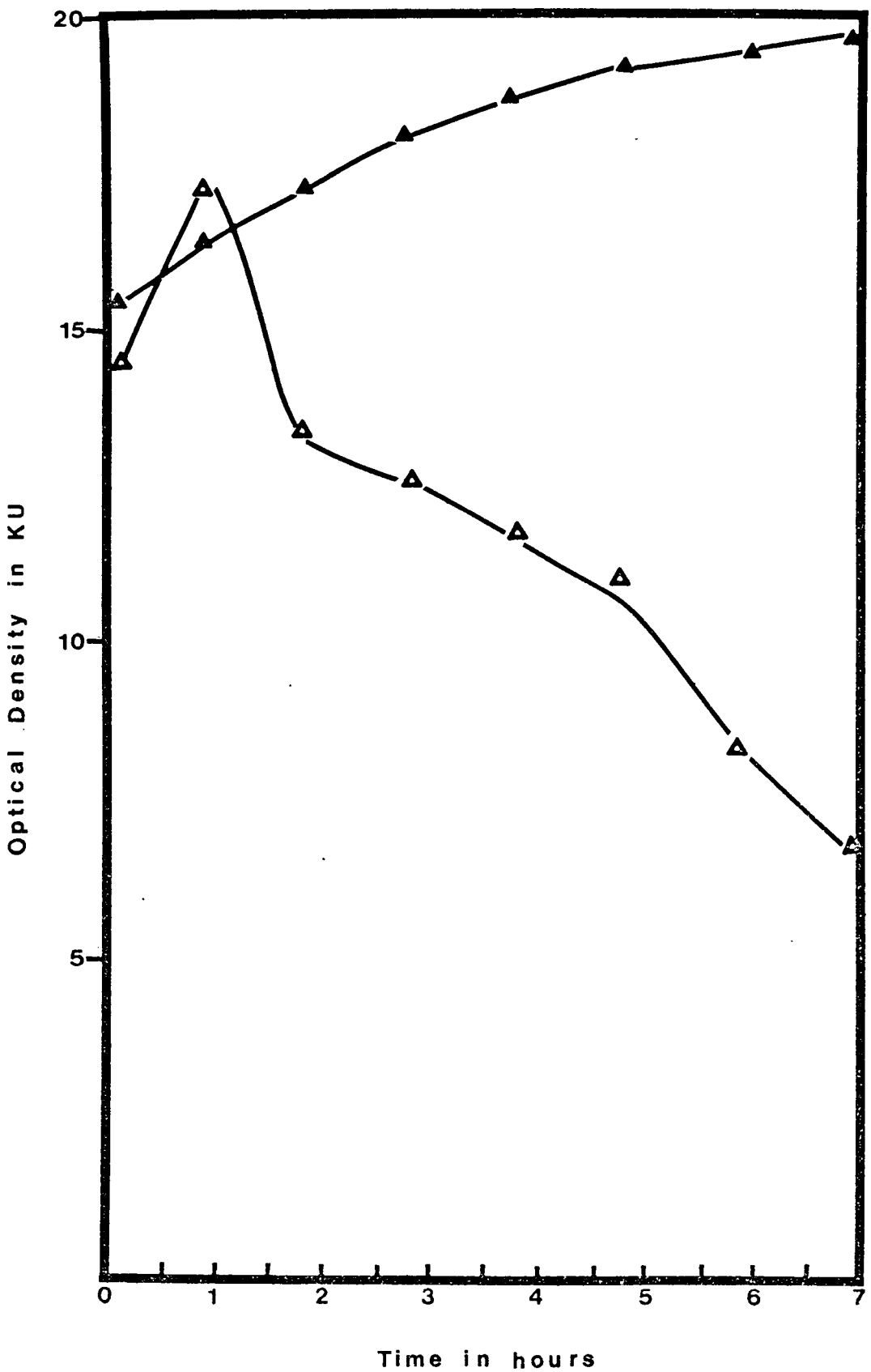
Figure 5

Effect of UV irradiation on strain C-328

and E. coli K-12 (λ)



Effect of UV Irradiation on C-328 and *E. coli* K-12



minutes (Figure 6). A decline in the optical density was seen thereafter. When an indicator bacterium was challened with culture filtrate collected at 240 minutes P1 phage was detected. Therefore Thymine starvation induces P1 from strain C-328.

e. Chloroamphenicol

Chloroamphenicol (to give a concentration of 10 ug/ml) was added to a three hour old actively growing culture of C-328. There was a slight increase in the optical density for the initial sixty minutes (Figure 7). Thereafter no significant decrease or increase in the optical density was observed between 4 to 7 hours of incubation. The failure to observe a decrease or increase in the optical density of the culture was probably due to the inhibition of protein synthesis. The inability of protein to be synthesized affects the synthesis of RNA and DNA (Lark, 1972) thereby causing stasis of the culture which was observed by optical density measurements.

When culture filtrate collected at four and eighteen hours were spotted on an indicator bacterium no lysis was observed. The failure to detect P1 phage when C-328 was grown in the presence of chloroamphenicol was probably due to the absence of certain enzymes necessary for phage DNA synthesis and the inability to synthesize protein coat material necessary for complete phage (Hershey and Melechen, 1957). P1 phage was not induced in the presence of chloroamphenicol.

Figure 6

Effect of thymine starvation on strain C-328

The Effect of Thymine–Starvation on Strain C–328

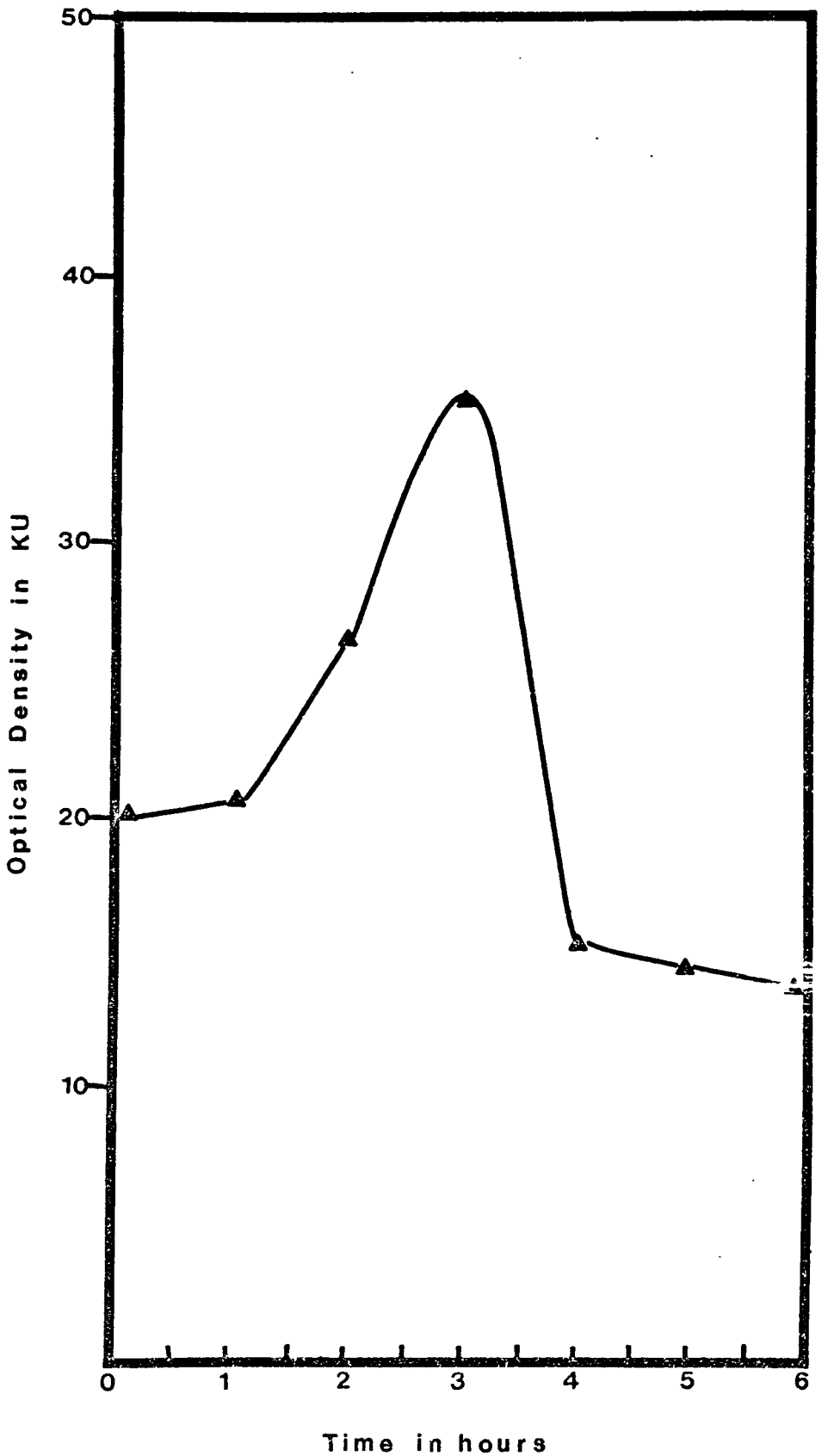
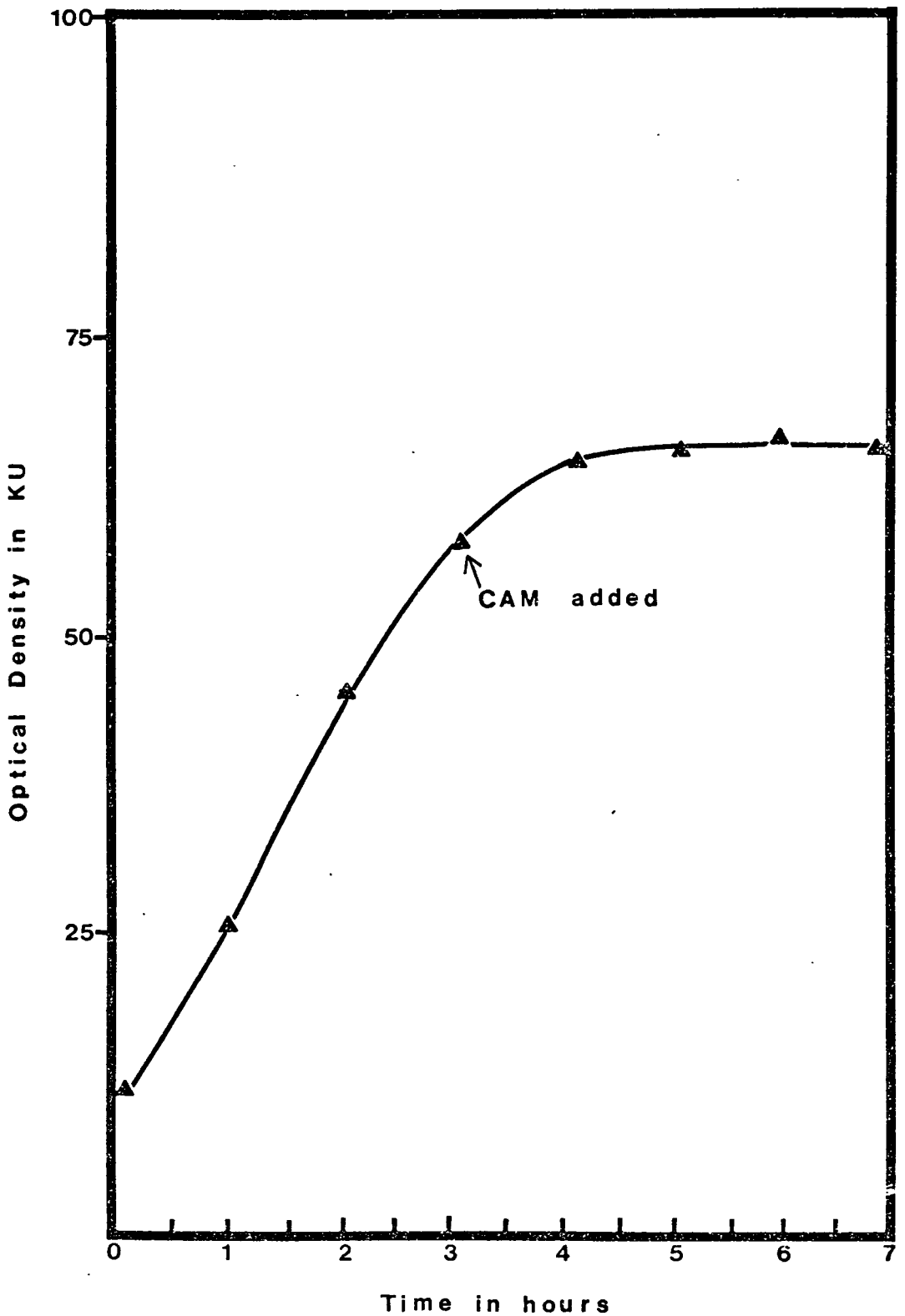


Figure 7

Effect of chloroamphenicol (10 ug/ml)

on strain C-328

Effect of Chloroamphenicol on Strain C-328



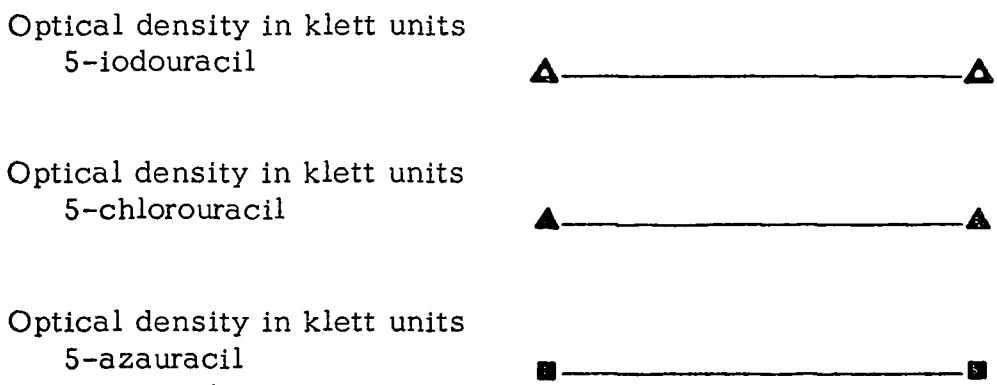
f. Uracil derivatives

Several derivatives of uracil were chosen to determine if they could induce P1 phage from C-328. The uracil derivatives 5-iodouracil and 5-chlorouracil are analogs of thymine, being incorporated into DNA (Matthews, 1958). The uracil analog, 5-azauracil, has been reported to inhibit RNA synthesis in E. coli 15T$^-$ by Chihak and Sorm (1964). The biological activity of certain uracil derivatives (5-carboxylic acid uracil, 5-acetyluracil and 5-aminouracil) on organisms have not been described.

Each of the uracil derivatives mentioned above was substituted for thymine in the MS-7 medium. The final concentration of each uracil derivative was 10 ug/ml. Strain C-328 was incubated for seven hours in the presence of these compounds. The optical density of all cultures increased at least twofold for the first three hours (Figures 8 and 9). After this time the optical densities decreased steadily in each of the cultures with the exception of 5-aminouracil. The optical density of this culture remained the same for an additional hour before declining. Lysis of the indicator organism was observed with culture lysates of all cultures at four hours of incubation. These results showed that uracil derivatives thymine analogs and uracil analogs could induce P1 from its prophage state.

Figure 8

Effect of uracil derivatives on strain C-328



Effect of Uracil Derivatives on C–328

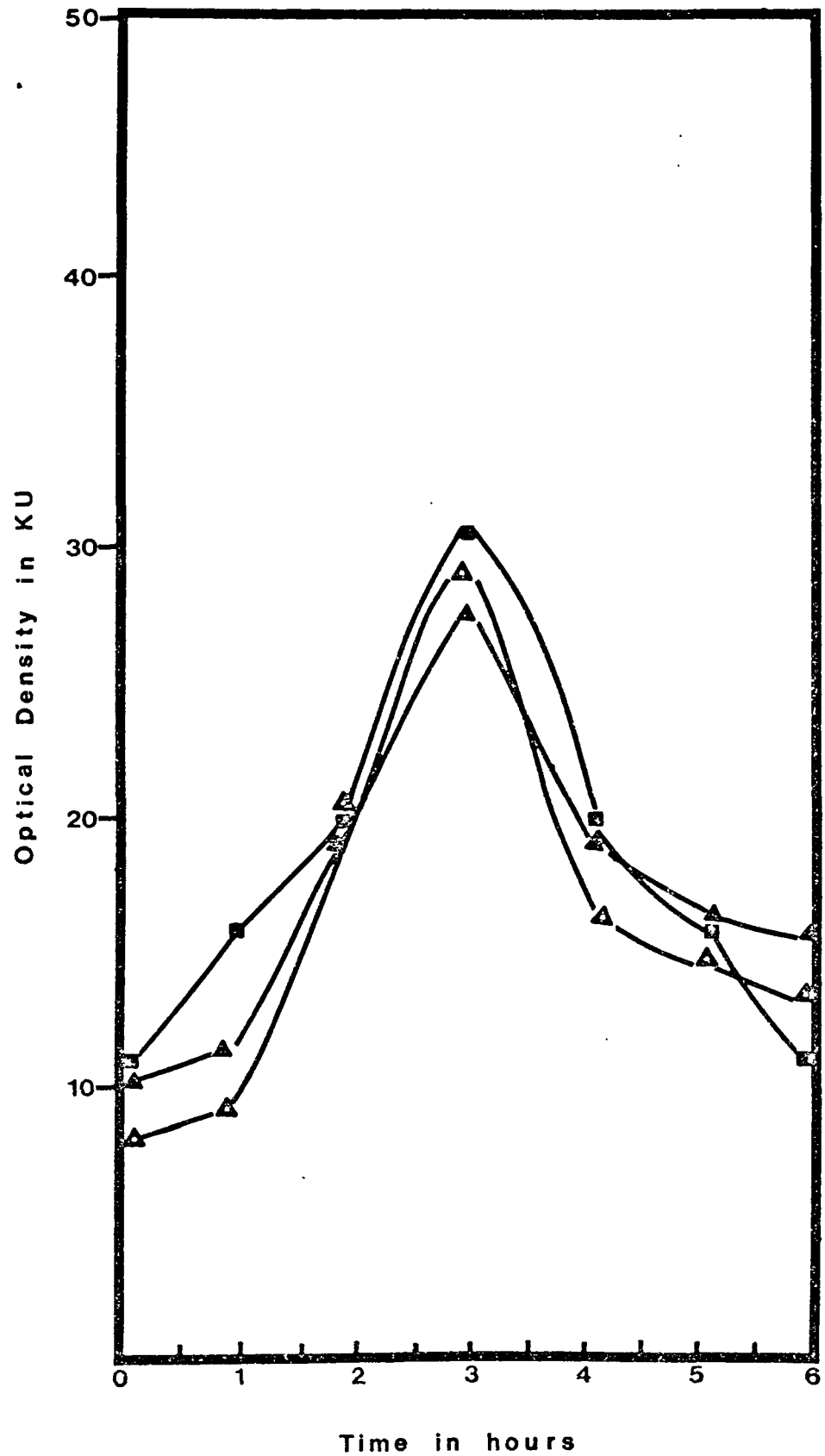
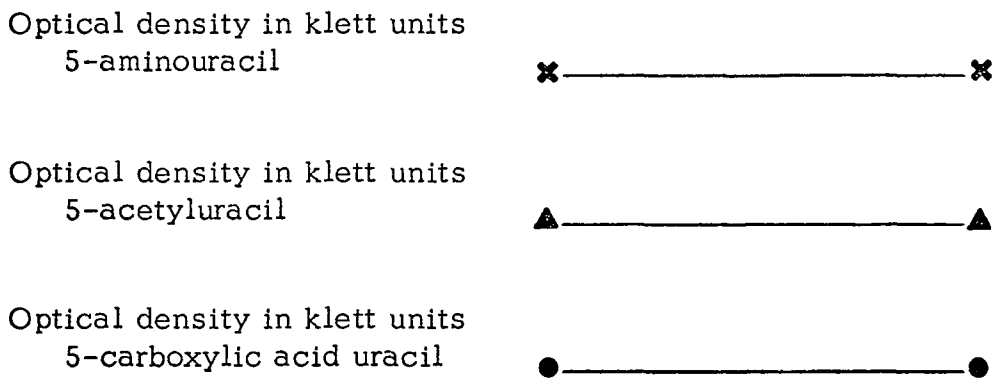
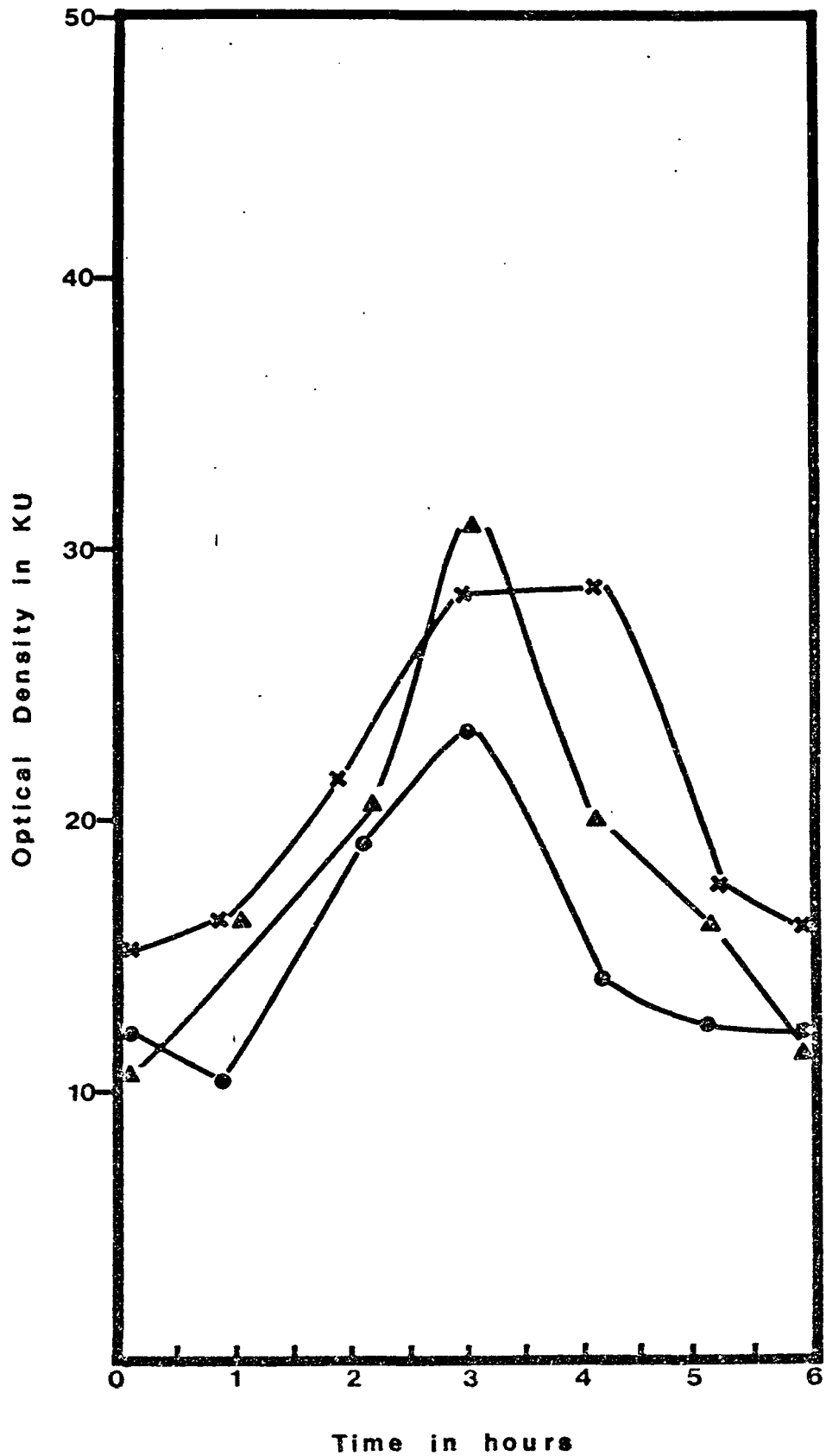


Figure 9

Effect of uracil derivatives on strain C-328



Effect of Uracil Derivatives on C-328



Physical Factors

a. pH factor

MS-7 medium was adjusted to alkalinity or acidity by adding 1 N NaOH and 1 N HCl respectively, in appropriate quantities to yield the desired pH. A preliminary experiment revealed that pH 4 and below, and 10 and above, failed to support growth of strain C-328. The optical density readings of the cultures at pH 5 through 9 are shown in Figure 10. A continuous increase in the optical density readings for all cultures was observed for seven hours of incubation. When culture filtrate from each culture was spotted on indicator organism lysis did not occur.

b. Osmotic pressure

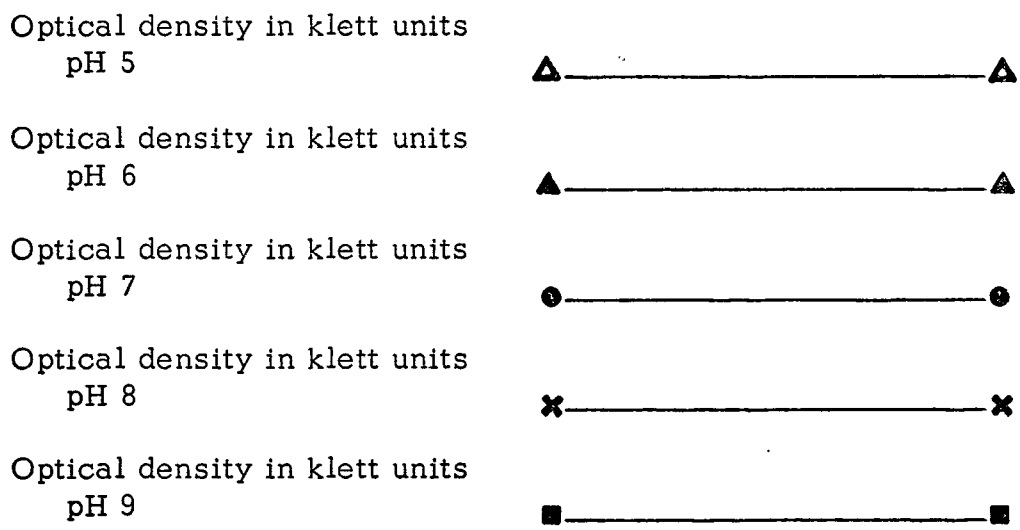
Strain C-328 was inoculated into MS-7 medium containing 0.33 M sucrose. There was a twofold increase in the optical density of the culture during the initial 60 minutes (Figure 11). No significant increase or decrease in the optical density was observed during six hours of incubation. Culture filtrate from three and six hours did not lyse the indicator organism.

c. Elevated temperature

A plot (broken line curve) of the optical density readings of strain C-328 grown in MS-7 medium at 45°C is shown in Figure 12. This

Figure 10

Effect of different pH on C-328



Effect of pH on C-328

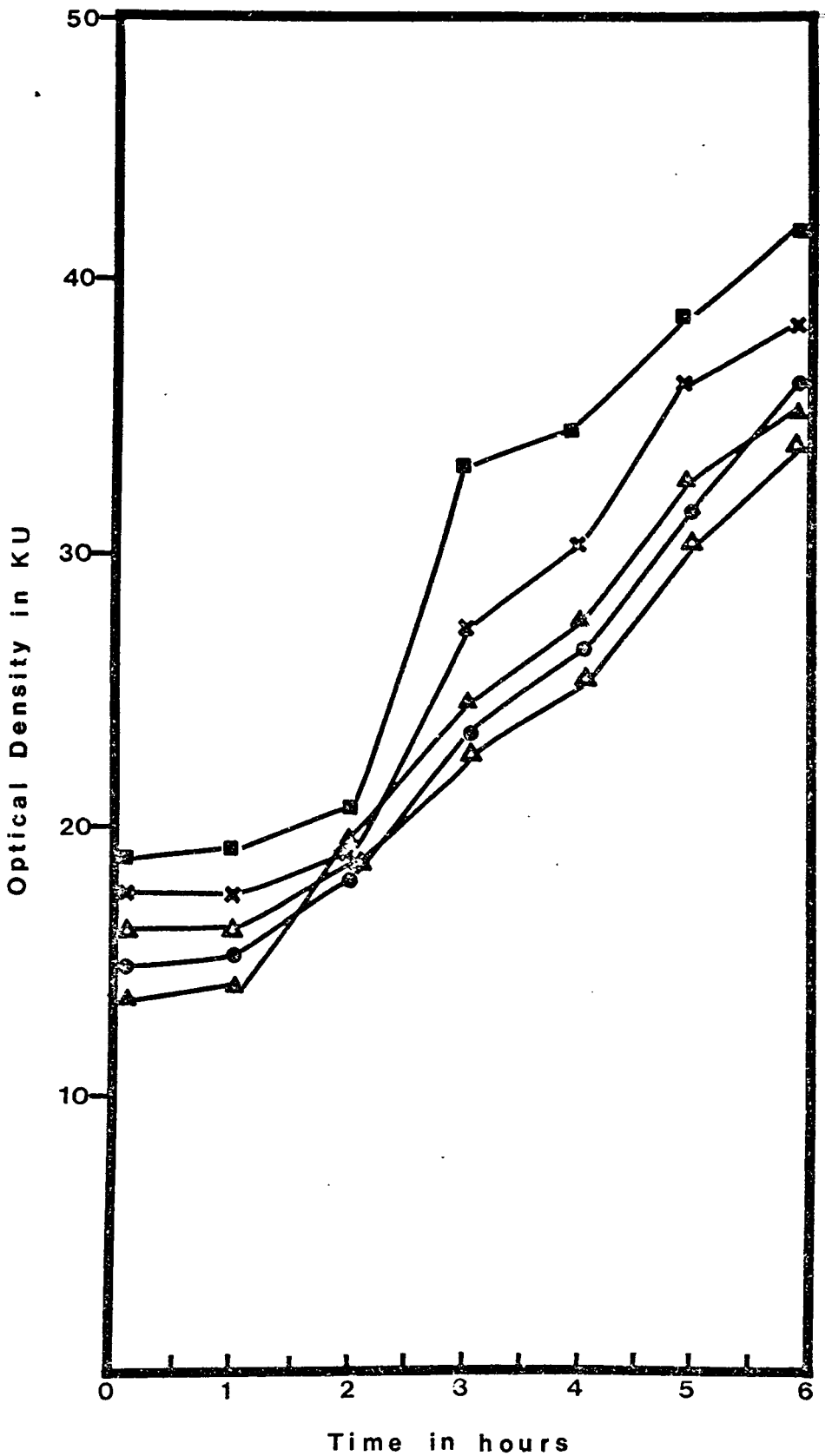


Figure 11

Effect of high osmotic pressure on strain C-328

Effect of High Osmotic Condition on Strain C-328

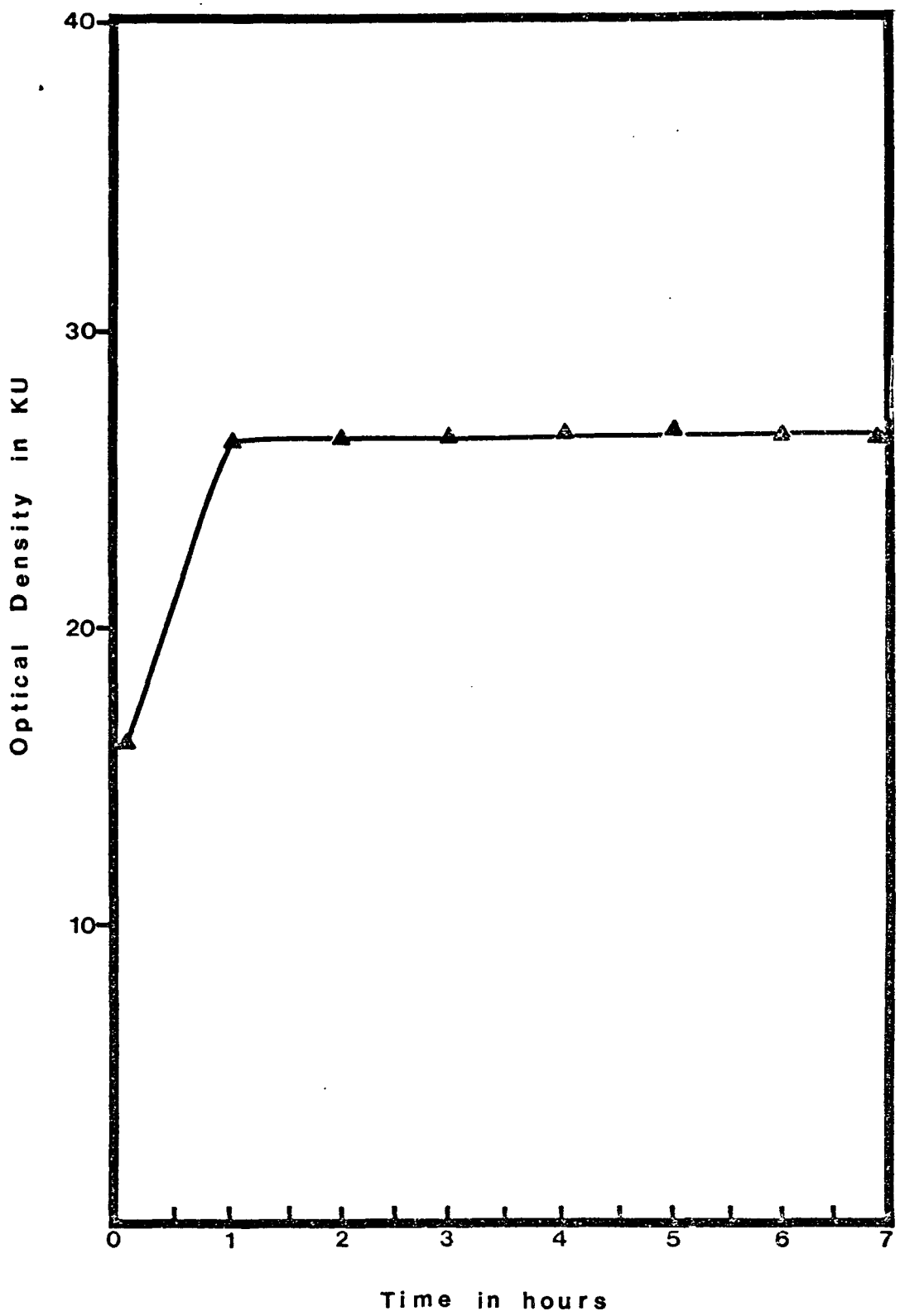
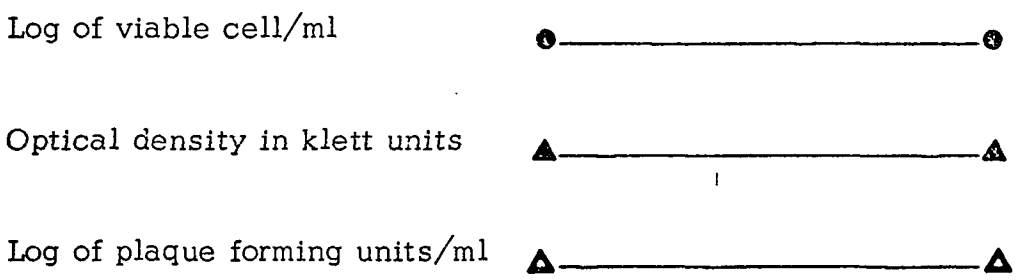
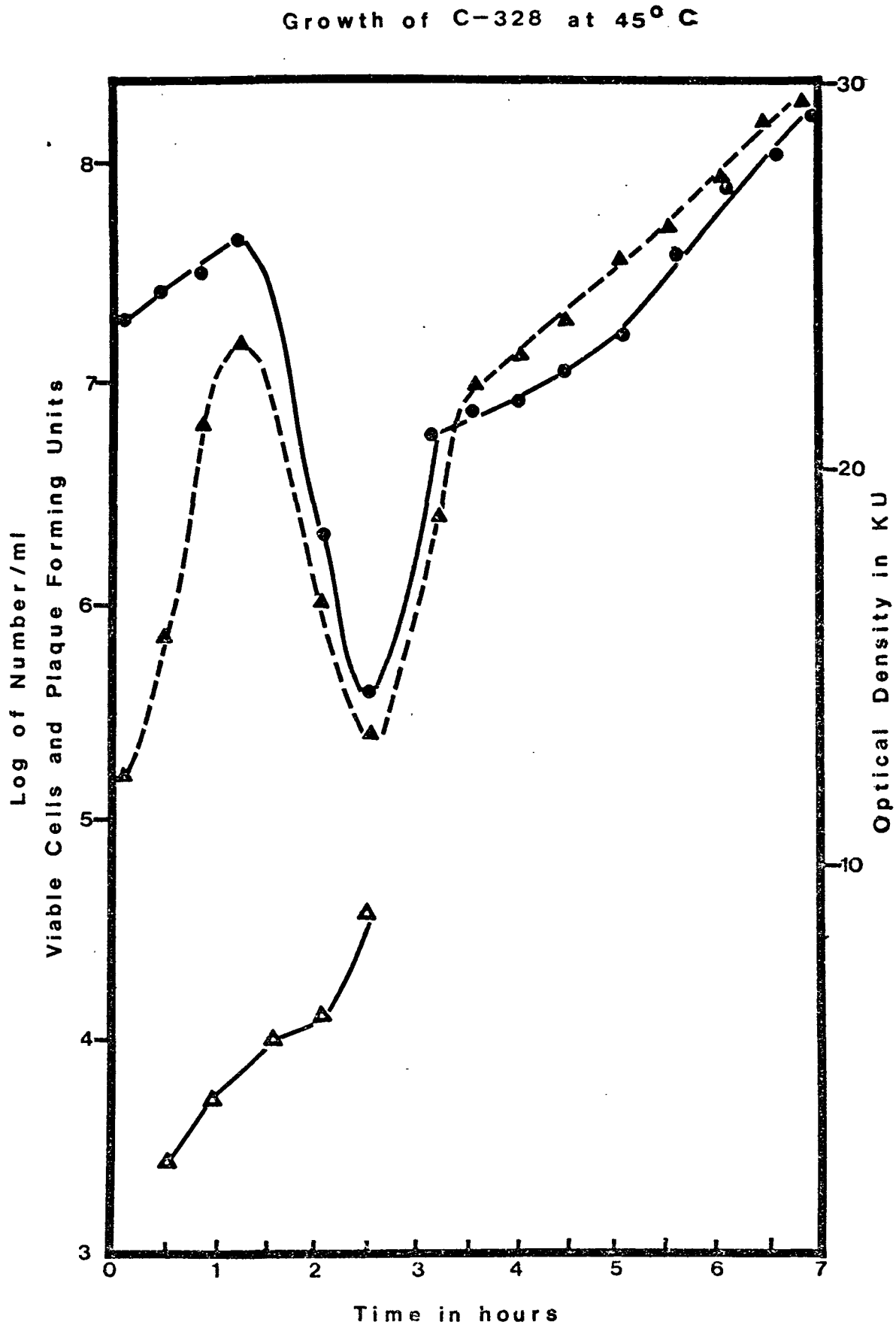


Figure 12

Growth of C-328 at 45°C




Growth of C-328 at 45° C
Log of Number/ml
Viable Cells and Plaque Forming Units
Optical Density in KU
Time in hours
8
7
6
5
4
3
30
20
10
0
1
2
3
4
5
6
7

growth curve revealed a bimodal growth response with the initial maximum optical density, 24 klett units, occurring at 90 minutes. The decline in optical density to 15 klett units occurred at 150 minutes of incubation. The continuous increase in optical density to 30 klett units, that followed the decline phase, was observed for the remaining incubation time. P1 phages were detected in the culture filtrate between 30 to 150 minutes only. This experiment clearly showed that strain C-328 would undergo phage induction and lysis when grown at 45°C.

Thermoinduction of P1 Phage

The bimodal growth response of strain C-328, shown in Figure 12, at 45°C suggests that phage induction and lysis of a large number of bacteria cells did not occur. When the growth response was measured by viable cell count a bimodal growth curve (continuous line), similar to that obtained for optical density readings, was obtained. These results are shown in Figure 12. The absence of P1 phages in the culture filtrate after 150 minutes was probably due to no phage liberation after 150 minutes and those phages that were liberated earlier had been readsorbed by the bacteria cells which continued to multiply.

The bimodal growth response and the presence of P1 phages in the culture filtrate between 30 and 150 minutes suggest that certain cells of the culture underwent phage induction and lysis whereas other cells

failed to undergo phage lysis continuing to multiply at 45°C.

Isolation of Thermoinducible Phage

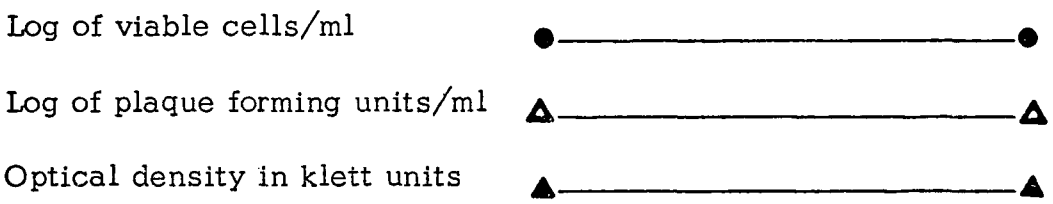
The results obtained from incubating C-328 at 45°C caused the investigator to suspect that this culture contains bacteria cells that were lysogenized by two phage types. One of the phage types being sensitive to thermoinduction whereas the other phage type resisted phage induction at 45°C. Previous results show these two phage types present in strain C-328 were inducible by chemical agents. To investigate the suggested possibilities on thermoinducibility of certain bacterial cells of C-328 experiments were designed to isolate the two lysogens.

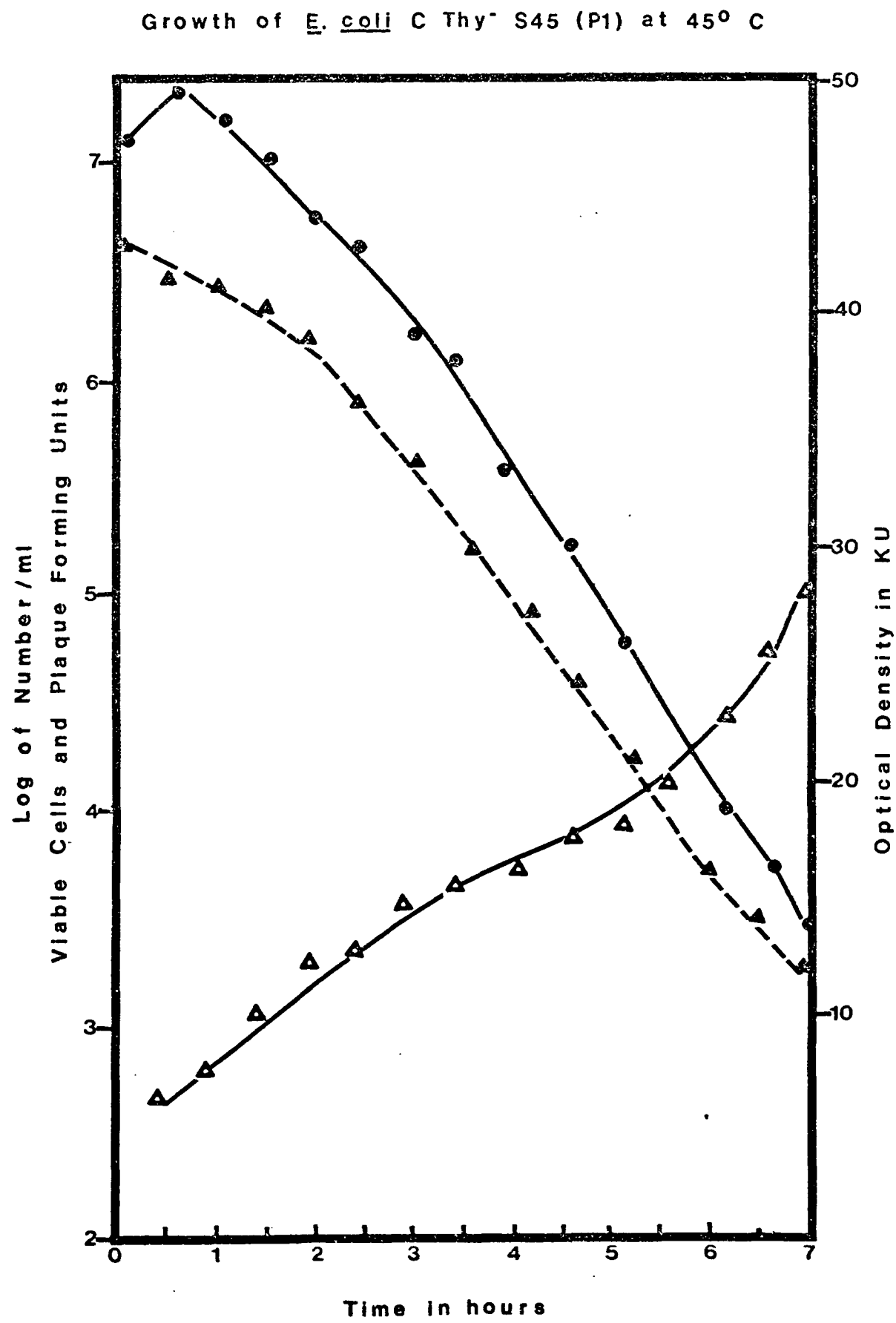
To obtain cells containing the thermoinducible phage a culture of C-328 was incubated in MS-7 medium at 45°C. At thirty minutes following the initial decline in optical density a 20 ml sample was collected from which bacterial cells were removed using a sterile millipore membrane filter. The phages present in this culture filtrate were used to lysogenize E. coli C thy-321 to give rise to the lysogen designated as E. coli C Thy⁻S45 (P1).

Figure 13 illustrates the growth response when E. coli C Thy⁻S45 (P1) was incubated at 45°C for seven hours. The presence of phages were detected after thirty minutes. The continuous increase in the number of phages liberated correlated with the continuous decline in the optical density and viable cells.

Figure 13

Growth of E. coli C Thy^{-}S45 (P1)




Growth of E. coli C Thy⁻ S45 (P1) at 45° C
Log of Number / ml
Viable Cells and Plaque Forming Units
Optical Density in KU
Time in hours
2
3
4
5
6
7
10
20
30
40
50
0
1
2
3
4
5
6
7

To isolate the non-thermoinducible lysogen a culture of C-328 was incubated at 45°C for 150 minutes. Bacterial cells were harvested from 20 ml of a culture sample, using sterile millipore filter, and washed before resuspending in 20 ml of MS-7 solution. This organism, non-thermoinducible, was obtained in pure culture by streaking on a nutrient agar plate.

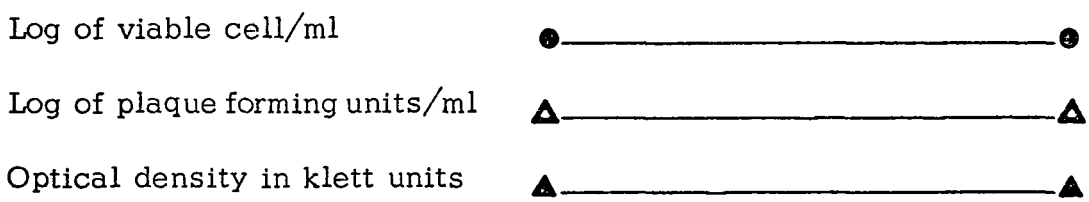
Figure 14 illustrates the growth response of this organism when incubated at 45°C. A continuous increase in the optical density and viable cells was observed for seven hours. At 120 minutes the growth rate decreased slightly. This probably reflects a reduced growth rate which will occur when a bacterium is grown above or below its optimal temperature for growth. No phage was detected in culture filtrates taken at 120, 240 and 420 minutes of incubation.

When both of the isolated lysogenic strains, derived from C-328, were incubated in BU-medium for 18 hours phages were detected in both culture lysates. The non-thermoinducible lysogen was designated E. coli C Thy⁻R45 (P1).

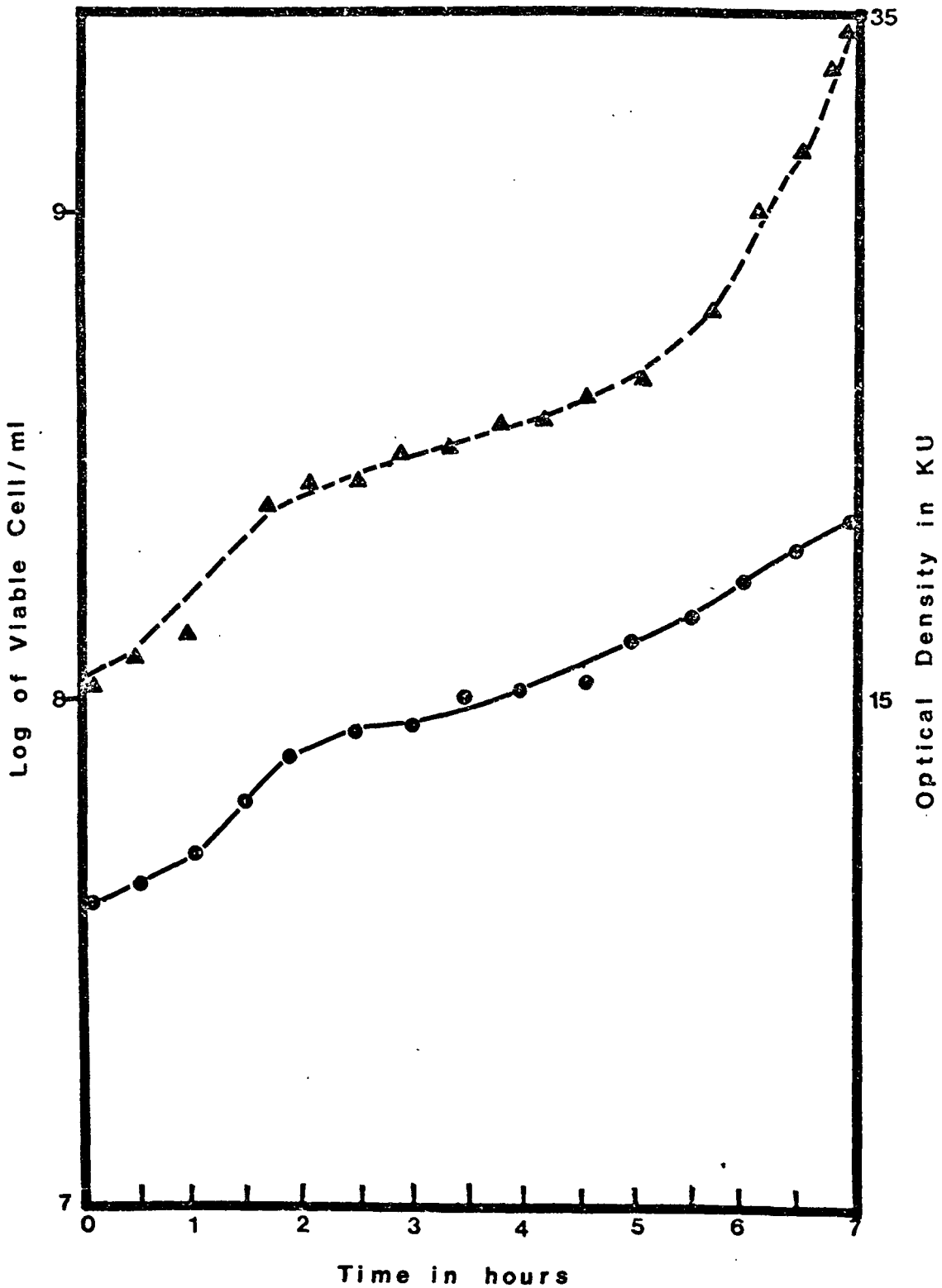
When the plaque morphology of the phages of the two lysogens that developed on E. coli C were compared, the plaques of E. coli C Thy⁻S45 (P1) were large (1 mm in diameter) smooth edge type whereas the plaques of E. coli C Thy⁻R45 (P1) were smaller (0.3 mm in diameter) with smooth edges.

Figure 14

Growth of E. coli C thy$^-$ R45 (P1)



Growth of E. coli C Thy⁻ R45 (P1) at 45° C



These experiments confirmed that E. coli C thy-3284 (P1) contains two lysogens each carrying a different phage. One of the lysogens was thermoinducible whereas the other was not. Both lysogens were induced by 5-bromouracil.

Host Range of Induced Phages

To determine if there were any differences in the host range of the induced phages, lysate from each was spotted on bacteria strains listed in Table II. The host range of the three phage lysates were the same. None of the P1 lysogens for each of the phages was lysed when challenged with its or the other lysates. Escherichia coli C thy-3284 (P1) was not lysed, probably because it was the parent lysogen. Resistance to phage lysis by the E. coli C Thy$^-$ S45 (P1) and E. coli Thy$^-$ R45 (P1) to the three phage lysates may indicate a common phage immunity by a host bacterium carrying either phages in the prophage state. The difference in thermoinducibility and plaque morphology of the two isolated lysogens indicates a possible mutation of these two characters of one of these phage types to give rise to the second phage type.

The host range study showed that the thermoinducible P1 phage had the same host range as the non-thermoinducible P1 phage on the organisms tested neither differing from that of the BU induced phage of E. coli C thy-328 (P1).

TABLE II

Bacteria Strain	Sensitivity to Phage P1	R45(P1)	S45(P1)
E. coli C thy-3284 (P1)	-	-	-
E. coli C Thy$^-$R45 (P1)	-	-	-
E. coli C Thy$^-$S45 (P1)	-	-	-
E. coli C thy-321	+	+	+
E. coli C 600 (21)	+	+	+
E. coli K-12 Met$^-$, thy$^-$3291 (λ)	+	+	+
E. coli K-12 (434)	-	-	-
E. coli K-12 Thy$^-$his-334	+	+	+
E. coli K-12 Thy$^-$, his$^-$159	+	+	+
E. coli K-12 Ura$^-$, Pan$^-$, His	+	+	+
E. coli K-12 Try$^-$ 444	+	+	+
E. coli B	-	-	-
E. coli B Met$^-$	-	-	-
E. coli B/r	+	+	+
E. coli 15T$^-$	+	+	+
E. coli W 3104 (λ)	+	+	+
E. coli 3704	+	+	+

DISCUSSION

The induction of P1 phage from E. coli C thy-3284 (P1) by thymine analogs was expected since Melechen (1964) found 5-bromouracil to induce P1 from E. coli B3. Experiments were designed to determine if thymine analogs themselves were unique in their ability as a phage inducing agent for E. coli C thy-3284 (P1). The induction and lysis of P1 phage by other uracil derivatives, such as 5-azauracil; an RNA inhibitor and 5-aminouracil; whose mode of action was unknown, showed that direct interference with DNA synthesis was not the only initial sensitive site for P1 induction.

Failure of UV irradiation to induce P1 phage was consistent with similar observations by Bertani (1958). The lambda phage system was inducible by UV irradiation. It is known that the lambda chromosome is in physical contact with the host chromosome and its induction is sensitive to several agents that interfere directly with DNA synthesis (Campbell, 1962). There is sufficient evidence about the P1 phage being different from lambda phage in that it is an episome with a extrachromasomal location (Ikeda and Tomizawa, 1968; Boice and Luria, 1963; Scott, 1970a). This inability of the P1 lysogen to being induced reveals another difference that exists between it and the lambda phage. Thymine starvation is known to induce lambda phage (Korn and Weisbach, 1962)

but there is need for thymine to be added to the lambda phage system before lysis will occur. Thymine starvation caused P1 phage induction and phage lysis in the absence of thymine, thereby revealing another difference between the two phages. The induction of P1 by thymine starvation is consistent with the observations made by Melechen and Skaar (1962). Hayes (1968) stated that differences in prophage induction, of different phages, was a function of the genome of the phages and not associated with its bacterial host. This investigator suspects that one significant difference is associated with whether the prophage is attached or inserted into the host chromosome. The lambda phage is known to be incorporated into its host chromosome (Campbell, 1962), therefore the prophage state of P1 may resemble the "attachment" model described by Hayes (1968).

The significance of the failure of the increased osmotic pressure and altered pH conditions to induce P1 phage is not apparent at this time. The bimodal growth response of the P1 lysogen when incubated at 45°C was not expected. Scott (1970b) observed thermoinduction of several P1 lysogens of E. coli. Rosner (1972) used a thermoinducible P1 system to transfer the chloroamphenicol marker in E. coli. Both investigators failed to observe a bimodal growth curve. The significance of the observed bimodal growth response was found to be associated with two phage types. The thermoinducible phage differed morpho-

logically from that of the non-thermoinducible type in that it was approximately three times greater. The two morphological plaque types had been observed with the induction of C-328 with 5-bromouracil but the significance of the two plaque types was not apparent in these earlier experiments. It could have been that E. coli C thy-3284 at some time had been lysogenized, accidently, with two phage types. The presence of more than one phage type in a culture of P1 lysogens has been reported by Bertani (1951), Lennox (1955), and Walker and Anderson (1970).

The results from the host range studies using the thermoinducible phage and non-thermoinducible phage suggest that both are P1 phages. This investigator therefore suspects that one phage type represents a mutation of the parent phage. Electron micrographs could give an added dimension in resolving the question of the emergence of a mutant P1 phage from its parent phage.

This study allows one to conclude that certain chemical and physical agents could cause phage induction and phage lysis of E. coli C thy-3284 (P1). The presence of two phage types were distinguishable morphologically and by being sensitive or resistant to thermoinduction. The presence of two phage types allows the investigator to postulate that the ability of P1 phage to be a generalized transducing phage may be due to the presence of two or more distinct P1 phage types present in a single bacterial strain, with one phage type per bacterium.

SUMMARY

This investigation has shown that analogs of thymine induced P1 phage from E. coli C thy-3284. The P1 phage was induced by the antibiotic Mitomycin C and thymine starvation. The induction of P1 phage from strain C-328 by uracil derivatives indicates that direct interference with DNA synthesis is not necessary for P1 prophage induction.

UV irradiation, which caused phage induction and lysis in the lambda system, did not induce the P1 phage from C-328. This suggests a difference between the two phage systems. Inhibition of protein synthesis by chloroamphenicol did not induce P1 phage from C-328. An alteration in certain physical conditions, pH and osmotic pressure failed to induce P1 phage.

When E. coli C thy-3284 (P1) was incubated at 45°C, a bimodal growth response was observed. The significance of this bimodal response was associated with the presence of two phage types: a thermoinducible and non-thermoinducible P1 phage in the culture of C-328. It was shown that E. coli C thy-3284 (P1) was lysogenized by two phage types, such that some cells were lysogenized by the thermoinducible phage while others were lysogenized by the non-thermoinducible phage. It is suggested that one phage type was probably a mutant of the parent phage.

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