INDUCTION OF PROPHAGE IN *BACILLUS SUBTILIS* 168 (ϕ105)

by 4689

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INTRODUCTION

Lysogenic bacterial strains may produce phage after they are exposed to small doses of ultraviolet light. This phenomenon was first found by Lwoff et al., in B. megatherium im 1950. Since then it has been found that a number of physical and chemical agents could induce phage production in lysogenic bacteria. Among them are soft X-rays(Latarget, 1951), organic peroxides (Lwoff & Jacob, 1952), nitrogen mustard(Jacob, 1953), irradiated leucovorin (Borek, et al., 1952) and L-azaserine(Mudd, et al., 1955). Both genetic factors (Weigle, et al., 1951) and physiological factors(Jacob, 1952) appear to control the induction of prophage by UV light. Still, very little is known about the mechanism by which the inducing agents act. It has been shown that the primary effect of an inducing agent is on the bacterial component but not on the prophage component of the lysogenic system(Marcovivh, 1956). In the biochemical study of an induced lysogenic system of B. megatherium 889(1), by UV light, Siminovitch showed that RNA synthesis continued, and that there was some residual cell growth. However, the synthesis of DNA was blocked during the first third of the latent period. He suggested that such bacterial disturbances caused the prophage to enter the vegetative state. In their repressor hypothesis, Jacob & Monod(1961) argued that in the lysogenic system , vegetative phage replication was prevented by cytoplasmic repressors similar to those postulated in the control of protein synthesis.

Thymine deprivation of a thymineless auxotroph leads to the induction of phage in E. coli(λ)(Korn & Weissbach, 1962) and of P1b prophage in E. coli B 3(P1b) (Melechen & Skaar, 1962). Also mitomycin C, which has been shown to inhibit bacterial DNA synthesis (Shiba, et al., 1959), can induce prophage development in E. coli K-12 (λ) (Takagi, et al., 1959) and also in Bacillus

subtilis W 168 (ϕ105) (Rutberg, et al., 1969). Both of these (thymine starvation and mitomycin C) inhibit DNA synthesis. This suggests that interruption of DNA synthesis in the host cell may cause prophage induction.

In the work presented here nalidixic acid has been selected to study the phenomenon of induction for two reasons: first, nalidixic acid was shown to inhibit DNA synthesis in both B. subtilis and E. coli (Goss, et al., 1964; Cook, et al., 1966), and second, nalidixic acid caused a specific loss of newly replicated DNA in B. subtilis (Rammareddy & Reiter, 1969). The purpose of this work is to investigate the induction of B. subtilis 168 (ϕ105) by nalidixic acid and to test whether there is any relation between the position of replicating point and prophage induction.

LITERATURE REVIEW

Lysogeny

A lysogenic bacterium is a bacterium possessing and transmitting the power to produce bacteriophage(Lwoff, 1953., Bertani, 1953). In most cases, the lysogenic bacteria, obtained by infecting with lysogenic phage, possesses the same properties as the original non-lysogenic strains. Lysogenic bacteria had two important characteristics. One is the ability to produce phage as a stable, heritable trait, and the other is immunity to lytic infection by the same or closely related phages(Bertani, 1953). Thus, the presence of a given prophage in a cell prevents both multiplication and lysogenization by homologous infecting phage.

Immunity is an expression of the presence, in the bacterium, of a prophage homologous to the infecting phage. When the prophage, either spontaneously or after infection, enters the vegetative state, immunity disappears and the superinfecting phage start multiplying(Bertani, 1953; Jacob, et al., 1953). When inducible lysogenic bacteria are first induced and then infected with a mutant of the homologous phage, each bacterium releases particles of phages as well as of mutant phage. Immunity reflects a block in the replication of the nucleic acids of the superinfecting phage, but not a failure of the phage to adsorb or to infect its nucleic acid. Jacob & Monod (1961) reported that immunity was caused by the action of a specific repressor produced under the control of a phage genome. Mutation in this gene was thought to destroy the ability to produce a repressor and to render the prophage unable to establish or maintain lysogeny.

The physical association between prophage and bacterial genome has been demonstrated by genetic recombination in E. coli. For example, if a non-lysogenic male bacterium conjugates with a female lysogenic strain, some of the recombinant bacterial progeny may be non-lysogenic. If the male is lysogenic for X-phage and the female for Y-phage, some of the bacterial recombinant are lysogenic for X-phage, others for Y-phages; none will have both. Thus a prophage behaved as a bacterial genetic marker and could be mapped by its specific linkage with other markers. In this way, Jacob & Wollman (1961) mapped many prophage sites in the chromosome of E. coli.

Many alternative modes of prophage attachment to the bacterial genome have been suggested. The most convincing one was that proposed by Campbell (1962). He suggested that, upon lysogenization, the phage genome circularized and integrated into the host genome by a crossover between homologous regions in the phage and host genome. Thus, as a result of mating between lysogenic bacteria that carried prophages differing in several genes, a linear map could be constructed in which the prophage genes were inserted between bacterial genes. For example, the location of prophage ϕ105 has been mapped between the bacterial markers phe-1 and ilv-A1 in lysogenic Bacillus subtilis 168 (Rutberg, 1969).

Spontaneous Production of Bacteriophage

In studying the lysogenic strain of E. coli, Bertani (1951) found that during the middle period of the logarithmic phase of growth, the ratio of bacteria to free phage was constant, but the ratio changed at the beginning and at the end of the logarithmic phase of growth. He suggested that the ratio of free phage to bacteria depended on the physiological conditions

of the bacteria. He also showed that phage production by lysogenic bacteria was a discontinuous process, consisting of the sudden burst of many phage particles, instead of a continuous process such as a secretion of one phage particles after another from growing cells. The frequency of this spontaneous lysis in lysogenic cultures in each generation varied from $1:10^2$ to $1:10^6$. Thus, it seems that, in a culture of lysogenic bacteria, the development of prophage into phage occurred spontaneously in a small constant fraction of the growing cells.

Induction of Prophage in Lysogenic Bacteria

Lwoff et al.,(1950) found that UV light induced phage production and that essentially the whole induced lysogenic population lysed. They found that after UV light exposure, bacterial growth continued for a period corresponding to one or two generations. No phages were produced during this period. At the end of this period, cell lysis began. Jacob & Wollman(1953) compared the phage development in newly-infected, sensitive bacteria and in induced, lysogenic bacteria. They observed that the characteristics of phage development, such as latent period, and the average burst size, were similar in both systems. Thus the processes of phage multiplication were identical in induced lysogenic bacteria and infected, sensitive bacteria. It seems that the only dissimilarity occurs in the latent period immediately following irradiation and leading to the vegetative state from the prophage state.

There are certain lysogenic strains that do not produce normal phage after being treated with inducing agents. In these strains, phage development seems to be abortive in that most of the induced bacteria do not liberate phage particles upon lysis. Lwoff et al.,(1951) found a Bacillus megatherium

in which only one in 10^5 bacteria released phage spontaneously instead of one in 10^2 observed in the corresponding normal strain. After suitable irradiation, more than 90 per cent of the bacteria lysed but phage production did not increase at all. A similar phenomenon was found in various other species such as Pseudomonase pyocyanea or Salmonella typhimurium in which UV irradiation induced lysis, but only a small fraction of the lysed bacteria released mature phage.

Factors Controlling Induction

Bertani(1951) in his early study of the kinetics of phage production during the growth of bacterial cultures, revealed that the rates of phage production and of bacterial growth were not always parallel. This strongly suggested that phage production was controlled by external factors, i. e. some changes of the medium induced by bacterial metabolism. Two factors have been suggested to control phage production in lysogenic bacteria. The first is a genetic factor. After the discovery of UV induction, it appeared that in each bacterial species some systems were inducible whereas others were not. For example, inducible strains, E. coli K-12(Delbruck, et al., 1951) and non-inducible strains E. coli Lisbonne(Bertani, 1951) existed within the same species. With phages of B. megatherium studies by Ionesco(1951) it turned out that, a) when a bacterium was lysogenized with a phage derived from an inducible strain, the new strain was inducible, b) when lysogenization was performed with a phage derived from a non-inducible strain, the new strain was non-inducible, and c) when a non-inducible strain was lysogenized with a phage from an inducible strain, the double lysogenic strain, after UV irradiation, produced only phage coming from the inducible strain. Similar results have been found by Jacob (1952) in Pseudomonas pyocyanea. From these

studies it was concluded that the inducibility was controlled in part by the properties of the prophage. It now appears that the inducibility is always controlled by the genetic constitution of the prophage. For example, in the bacteriophage λ system, the product of gene C_1 was a protein that prevented the expression of most λ genes(Lieb, 1969). In lysogenic system, inactivation of this repressor protein resulted in the synthesis of a new species of λ m-RNA, and was followed by excision and replication of the prophage and lysis of the host cells.

The second factor determining the induction of prophage is the physiological state of the host bacteria. The effect of nutritional factors on aptitude, i. e. the ability of bacteria to respond to irradiation, has been demonstrated in Bacillus megatherium 889 (1) (Lwoff, et al., 1951). It was observed that UV dose which could induce almost all bacteria grown in yeast extract could not induce bacteria grown in a synthetic medium. Disturbances in bacterial metabolism may affect aptitude markedly. Jacob(1952) found, in a population of Pseudomonas pyocyanea deprived of glucose or ammonia salt, that the proportion of bacteria able to produce phage, and the sensitivity to UV light of these bacteria were strongly reduced.

The effect of amino acid starvation on phage induction has been observed by Borek(1952). He found that methionine or leucine starvation in a methionine or leucine-deficient mutant of E. coli K-12(λ) strain before UV irradiation, produced a similar decrease of aptitude. He also reported that during amino acid starvation after UV irradiation, neither bacterial growth nor lysis was observed. However, upon readdiation of the lacking amino acids, bacterial

growth and lysis were resumed. It was, therefore, concluded that amino acid starvation had not suppressed the inducing effect but had stopped the development of the prophage. Changes in the composition of the medium, or in the cationic balance after irradiation, also prevented phage production. Lwoff (1951) found that when an irradiated culture of B. megatherium grown in yeast extract was transferred to a broth medium, prophage development did not occur but bacterial growth proceeded. Huybers(1953) found that phage development in lysogenic B. megatherium after UV irradiation did not take place when the medium was deficient in manganese, induction did not occur if manganese was added. He also found that cobalt, zinc and copper could suppress phage production, and that this suppression was reversed by manganese. Jacob et al., (1953) showed that UV induction might be reversed by exposing irradiated bacteria to visible light, but this photo-restoration did not take place after exposure to X-rays or nitrogen mustard.

Inducing Agents

Certain chemicals induced the development of prophage in lysogenic strains. Physical agents, such as UV light(Lwoff et al., 1950), X-rays(Laterject, 1951), and γ-rays(Marcovich, 1956) also induced lysogenic bacteria. Lwoff & Jacob (1952) found that organic peroxides, and ethyleneimines could also induce prophage development. Hydrogen peroxide also induced phage production in certain inducible strains(Lwoff et al., 1952). Smith(1953) found that bacterial lysis was induced in Salmonella thompson by nitrogen mustard, nitrogen gas, glutathione and sodium thiolacetate, and this induction might be inhibited by urethane or ascorbic acid. Gots et al., (1955) found that azaserine could induce prophage in E. coli K-12(λ). Borek et al.,(1955) also reported UV-irradiated leucovorin had a inducing effect on E. coli K-12(λ). This

irradiated-elicited inducing effect might reside in the pteridine moiety of leucovorin, since aminopterin has also been found to be an inducing agent on E. coli K-12 (λ) (Ben-gurion, 1962). Mitomycin C has also been reported by Takagi et al. (1959) and Rutberg (1969) to induce prophage development in E. coli K-12 (λ).

Two different kinds of evidence have been found to support the theory that the inducing agents were acting on the bacterial components, instead of the prophage of the lysogenic system. One came from the estimation of the size of the induction target by means of X-rays. Induction appeared to result from a single ionization and the size of the target found was too large to be the prophage itself, but is of the same order of magnitude as the whole bacterial nucleus (Marcovich, 1956). The other evience came from the analysis of lysogenic bacteria carrying two different, but related prophages which can simultaneously develop in the same bacterium. The results showed that there was an existence of a correlation in the production of both types of phage. Such a correlation is incompatible with the hypothesis of a change in the prophage itself as the primary event of induction. Therefore, development of the prophage appeared as a secondary effect, the primary event consisting of some alteration of the bacterium.

Mitomycin C and Its Mode of Action

Mitomycin C is an antibiotic isolated from Streptomyces caespitosus by Wakaki (1958). It has been shown to possess antibacterial activity against both gram positive and gram negative bacteria (Takagi, et al., 1959). Takagi et al. also demonstrated that this antibiotic selectively inhibited the synthesis of DNA without any effect on the synthesis of protein and RNA in

E. coli. The same result was found in Salmonella typhimurium(Levine, 1961). Takagi et al.,(1959) showed that mitomycin C could induce prophage in E. coli K-12(λ). Similar results have been found in Salmonella typhimurium(P 22) by Levine(1961), and by Marmur et al., (1964) in Bacillus subtilis(PBSX). Reich et al. (1961) showed that mitomycin C could inhibit E. coli growth and that there was a breakdown of DNA but not that of RNA and protein. Mitomycin C also caused the covalent cross-linking of the complementary DNA strains (Szybalski, 1963).

Levine(1961) noticed that DNA was selectively inhibited in Salmonella typhimurium by treatment with mitomycin C, but these infected cells, could produce active phage particles on infection with phage P 22. Upon infection of mitomycin C-treated E. coli with T_2, T_3 or T_5, cells could regain the ability to synthesize DNA as shown by Sekiguchi et al., (1959); the phage particles produced by these cells were, however, non-infective. These results may be interpreted to mean that DNA synthesis proceeds in phage-infected bacterial cells through a pathway which differs from that of non-infected cells and which is resistant to the action of mitomycin C. An alternative explanation may be that infection of the cells is followed by a massive induction of enzymes connected with the synthesis of DNA, and that the production of these enzymes is inhibited by mitomycin C. This may be the reason of the synthesis of non-infective phage particles as described by Sekiguchi et al. (1959). The finding that inhibition of DNA synthesis in cells treated with mitomycin C was restored by infection with phage is consistent with the fact that mitomycin C had little effect on the ability of the DNA to transform for a given marker.

Thymineless Death and Prophage Induction

Thymineless death, the loss by a thymine-requiring auxotroph of the ability to multiply after deprival of thymine, was first found by Cohen & Barner(1954) in E. coli 15 T^-. A number of different hypotheses have been put forth to explain this phenomenon. One of them was that prophage induction, as described by Melechen(1962). Weissbach et al.,(1962) reported that phage was induced in a lysogenic strain of E. coli K-12(λ) during thymine starvation. Production of λ phage in these cells was arrested by depriving the cells of carbon, nitrogen, or phosphorus. They also noticed that conditions which prevented thymineless death, i. e. chloramphenicol treatment or carbon starvation, also inhibited phage induction by thymine starvation. After 60 to 90 minutes of thymineless incubation, the non-viable cells in the culture were not capable of producing maximal phage titers. Since full induction required about 120 minutes, Weissbach et al. concluded that thymineless death and thymineless induction may not be necessarily interdependent.

Melechen & Skaar(1962) also found phage induction in E. coli B3(P1b) and E. coli K-12(λ) during thymine starvation. Since there was no indication of DNA synthesis during the period of thymine starvation, they suggested that, during the starvation period the cells might undergo only the first step of induction, i. e. the prophage diverted to vegetative replication. Since they found that this prophage diversion was stopped abruptly by treatment with chloramphenicol, it was then hypothesized that a critical condition for prophage diversion was that protein synthesis had to accompany the inition of DNA synthesis. It was suggested that thymine starvation, as well as other inducing agents, acted by uncoupling a protein bond between prophage and bacterial DNA due to the DNA: protein imbalance.

Rolfe(1967) reported the effect of actinomycin D on thymineless death at different times during the course of thymine starvation in B. subtilis S. He found that there was a critical time for the addition of actinomycin D to stop thymineless death, corresponding to 60-70 per cent survival. After that time the surviving fraction continued to lose viability in the presence of actinomycin D. He concluded that thymineless death in Bacillus subtilis involved at least two steps:(1) a step involving RNA and protein synthesis during thymine starvation, and (2) a subsquent step requiring continued thymine starvation but independent of RNA and protein synthesis. It has been found that in one of the phage C1 mutants the repressor was reversibly inactivated by heating, and upon cooling repression was restored (Gros & Banono, 1968). Sicard et al., (1962) postulated that thymine starvation caused the accumulation of an internal inducer substance. If the derepression produced by increased levels of inducer is reversible, then thymineless induction would require (1) RNA and protein synthesis during thymine starvation, and (2) accumulation of inducers sufficient to prevent rapid reversal of derepression upon re-addition of thymine. This hypothesis was the same as the hypothesis formulated by Melechen (1962) who found that protein synthesis had to accompany thymineless induction in E. coli B 3 (P1b). However, the prophage diversion as described by Melechen might involve a series of steps beyond those leading to cell death, at least one of which required the synthesis of a protein.

Cummings & Mondale (1967) showed evidence against induction as a cause of thymineless death. They found that E. coli K-12(λ) suffered thymineless death with the same kinetics as the E. coli K-12 non-lysogenic strain. Donachie & Hobbs(1968) compared two different strains, E. coli 15 T^-(coli 15^+), and E. coli 15 T^-(coli 15^-), which was cured of the defective prophage

coli 15. They found that both strains showed the same loss of plating viability during the period of thymine starvation. However, upon readdition of thymine, there was a decrease in optical density and in the number of viable coli 15^+ cells but not the coli 15^- cells. Therefore, they suggested that thymineless death was not itself caused by phage induction.

Many other mechanism have been suggested as the cause of thymineless death. These include unbalanced growth(Cohen & Barner, 1954), nuclear damage (Gallant & Suskind, 1961), single-stranded breaks in the DNA of thymine starved cells(Mennigmann & Szbalski, 1962) and colicin production. A correlation between colicin production and thymineless death was shown by Sicard & Devoret (1962). They suggested that thymineless death was due to the production of colicin. Luzzati & Chevallier(1964) and Mennigmann(1965) also found that thymine starvation induced colicin synthesis in thymineless colicinogenic strains. Mennigmann found thymine-deprivation of E. coli 15 T^- could induce the production of colicins, if thymine was added after thymine starvation. He concluded that thymineless death was at least partially due to the formation of colicin.

Nalidixic Acid andIts Mode of Action

Nalidixic acid(1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthridine-3-carboxylic acid) is an antibacterial agent synthesized by Lesher et al.,(1962). This agent was shown to be lethal for proliferating cultures of E. coli(Goss et al., 1954). Associated with this lethal effect was the formation of the elongated, serpentine forms. Goss et al. also found that nalidixic acid inhibited specifically the synthesis of DNA. Slight effects on protein and RNA synthesis was observed only at high nalidixic acid concentrations during

prolonged incubation with nalidixic acid. Deitz et al., (1966) found that protein and RNA synthesis were necessary for the lethal action of nalidixic acid. Although chloramphenicol and dinitrophenol, which uncoupled oxidative phosphorylation, could prevent the bactericidal action of nalidixic acid upon E. coli, in the presence of an inhibiting concentration of chloramphenicol, nalidixic acid prevented DNA synthesis in E. coli 15 TAU. Nalidixic acid was shown to cause the degradation of DNA in E. coli 15 TAU (Cook et al., 1966). This degradation could be arrested by the addition of dinitrophenol or chloramphenicol to the cells treated with nalidixic acid. Nalidixic acid has also been shown to be mutagenic, and to induce excess DNA synthesis in E. coli after its removal from the cells. Boyle et al. (1969) showed that nalidixic acid had no effect in vitro on the synthesis of DNA of E. coli. It had no inhibitory effect on DNA-polymerase, deoxyribosyl transferase and deoxyribonucleotide kinase activity. They suggested that this absence of inhibition might be a reflection of the inability of nalidixic acid to bind to the DNA primer, because of the absence of cross-linked DNA after treatment of nalidixic acid. Gage & Fujita (1969) demonstrated that nalidixic acid had little inhibitory effect on phage SP01-infected B. subtilis DNA synthesis. They found the SP01 DNA synthesized in the presence of high concentrations of nalidixic acid had density characteristic of normal SP01 DNA and that the DNA was packaged into viable progeny phage particles. The rate of SP01 DNA synthesis was reduced and bacterial lysis was delayed. Recently, Ramareddy & Reiter (1969) showed another effect of nalidixic acid. They showed that B. subtilis 168 cells degraded 20 to 30 per cent of their DNA in 3.5 hours of exposure to nalidixic acid and that this degradation appeared to proceed sequentially along the chromosome from the most recently synthesized DNA to older DNA.

All the inducing agents studied so far blocked DNA synthesis either directly or indirectly. The mechanism by which prophage development was initiated after treatment of lysogenic bacteria with an inducing agent is still obscure. However, it is clear that a disturbance of DNA synthesis is needed for prophage induction. Since protein synthesis has to accompany the inhibition of DNA in order for induction to occur, it would appear that prophage induction involves the upset of a delicate metabolic balance which is responsible for the stable relationship between repressor molecules and prophage genes. The known effects of nalidixic acid leads one to determine the effects of nalidixic acid on lysogenic strains of Bacillus subtilis, and to test whether phage induction may be caused by the nalidixic acid induced degradation of DNA near the site of prophage integration.

MATERIAL AND METHODS

Bacterial Strains and Phage: Bacillus subtilis 168 thy⁻ind⁻, Bacillus subtilis 168 thy⁻ind⁺, and Bacillus subtilis 168 thy⁺ind⁻ are from the collection of this laboratory. Bacillus subtilis 168 thy⁻ind⁻ ts-134 was obtained from Dr. B. Mendelson. Phage ϕ105 was originally isolated from soil in the laboratory of Dr. F. Reily, was provided by Dr. Rutberg. Lysogenic derivatives of Bacillus subtilis 168 thy⁻ind⁺ and Bacillus subtilis 168 thy⁻ ind⁻ ts-134, were isolated by picking some cells from the center of turbid plaques formed by plating ϕ105 on the respective strains.

Medium and Chemical: A minimal medium (prepared as described by Spizizen, 1958) contained 14.0 g K_2HPO_4; 6 g KH_2PO_4; 2.0 g $(NH_4)_2SO_4$; 1.0 sodium citrate; and 0.2 g $MgSO_4 \cdot 7H_2O$ (in grams per liter of distilled water). This medium will be referred to be as subtilis salts(SS). Stock solution of 50% glucose and 2% casein hydrolysate were prepared. One ml of glucose and two ml casein hydrolysate were added to 100 ml of sterilized SS. This will referred to as CH medium. Thymidine solution and tryptophan solutions were made at 2mg/ml concentrations in distilled water. 10 µg/ml of thymidine solution was added to CH medium and 40 µg/ml tryptophan in all this experiments. NBS agar plates were prepared by pouring the sterilized medium containing 8.0 g nutrient agar, 10 ml of 10 X SS and 15 g Difco agar per liter of distilled water. NA soft agar contains 8 g/ml Difco nutrient broth, 5×10^{-3} M $MgSO_4$ and 2×10^{-5} $MnCl_2$ at pH 7.2. Penassay broth was prepared by dissolving 17.5 g dehydrated Sacto-Penassay broth in 1000 ml distilled water. This was sterilized in the autoclave for 15 minutes at 15 lbs presure. Nalidixic acid (Trade name Negram) was a gift from Dr. Archer of the Sterling- Winthrop Corp. This was dissolved in distilled water at

a concentration of 200 ug/ml by raising the pH to 8.0 with sodium hydroxide. Thymidine was a product of Calbiochem. ^{3}H-thymidine with a specific activity of 6 C/mM was obtained from Schwarz BioResearch, N. Y.

Phage Assay: Phage assay was made by mixing a 0.1 ml sample of lysogenic culture with three to five drops of indicator bacteria in 2.5 ml of melted top layer NA soft agar and then pouring the mixture on NBS plates. All plating were made in duplicate. The plates were incubated overnight at the temperature indicated. Logarithmically growing B. subtilis H cells were used as indicator bacteria.

Growth of Bacteria: Bacterial strains were maintained on NBS plates and were renewed every two weeks. In all experiments, cells were grown overnight at 37°C(or 30°C for the temperature-sensitive mutant) in CH medium containing 10 µg/ml thymidine or 40 µg/ml tryptophan(or both) as required by the strains used. Continuous aeration was achieved by bubbling air through the culture. The overnight culture was diluted 100-fold in the morning with the same medium and then grown to logarithmic phase. Rapid filtration was achieved by using a Millipore device with 47 mm Schleicher and Schnell membrane filters(pore size 0.45 µ). The cells were washed with and resuspended in CH medium that had been warmed to 37°C(or 30°C for the ts mutant). The washed cells were aerated at the temperature indicated to starved for thymine. Assay for viable cells was done by taking samples at different intervals, diluting them in SS and plating on NBS plates in duplicate.

Incorporation and Assay of Radioactivity: ^{3}H-thymidine was used to label DNA synthesis. Incorporated radioactivity was assayed by adding trichloroacetic

acid(TCA) to a final concentration of 5% at 0°C for 45 minutes to 60 minutes. The material precipitated by TCA was collected on Reeve Angel glass fiber filters. The filters were dried and counted in a Packard Liquid Scintillation counter after adding 2 ml of 98% 2,5-diphenyloxazole(PPO) and 2% 1.4-bis-2-(5-phenyl-oxazolyl)-benzene(POPOP) (Parkard "Premix", 5 g per liter toluene). Corrections were made by subtracting the background radioactivity.

Induction Experiments: Lysogenic derivatives of Bacillus subtilis 168 were grown overnight. They were diluted 100-fold in the morning with the same medium and brought to early logarithmic phase of growth. UV-irradiated induction was performed by putting 5 ml of the culture under the UV lamp(which gave a measured dose of about 25 erg/mm^2/sec at the distance of 60 cm) and irradiated for 60 seconds with constant shaking. Nalidixic acid induction was performed by treating the culture with 40 µg/ml nalidixic acid. At the times indicated, nalidixic acid was removed by diluting with the same medium or by centrifuging the culture and resuspending the cells in fresh medium. Infectious centers were measured as described in the phage assay in a Klett-Summerson Photoelectric colorimeter using a red filter.

RESULTS

A. Induction of Bacillus subtilis 168 thy⁻(ϕ105) by UV-irradiation:

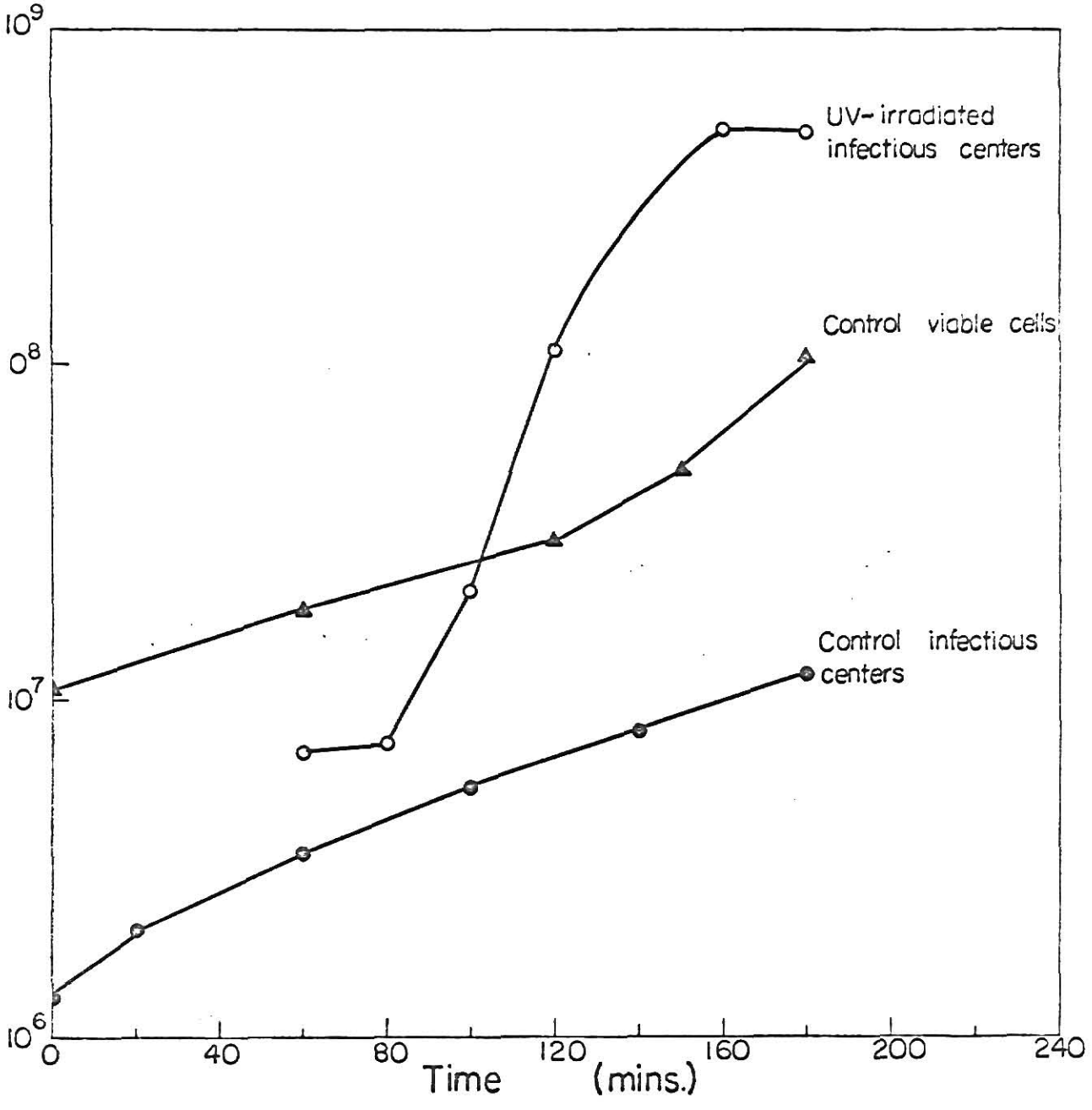
It has been shown that ultraviolet light is a potent inducer for phage development. To test the sensitivity of B. subtilis 168 thy⁻(ϕ105) to UV-irradiation, and for use as a standard for comparison with the data to be presented later, the following experiments were performed. Exponentially growing cells were washed and resuspended in CH medium plus 10 μg/ml thymidine and incubated for 60 minutes. A portion of the culture was then UV-irradiated for 60 seconds(25 ergs/mm/sec) . The cells were then incubated. At intervals, the culture(both UV-irradiated and control) was assayed for viable cells and infectious centers. Changes in optical density were also measured. Figure 1 shows that the spontaneous production of phage parallelled the rate of bacterial growth. Immediately after UV-irradiation, there was a three fold increase of phage production. The burst size was about 60 phages per cell. Figure 2 shows that phage production was accompanied by cell lysis which was measured by the decrease in optical density of the culture. The increase of cell turbidity for 60 minutes after UV-irradiation indicated that protein synthesis continued during the period of induction. This experiment clearly shows that Bacillus subtilis thy⁻(ϕ105) is UV-inducible.

B. Induction of Bacillus subtilis 168 thy⁻(ϕ105) by thymine starvation:

When B. subtilis 168 thy⁻(ϕ105) lysogenic cells were incubated in a thymineless medium, the cells underwent thymineless death in the same way as non-lysogenic thymineless auxotrophs(Fig. 3). In the absence of thymine, there was less than two fold increase of phage particles after 120 minutes of starvation. When thymine was added to the cells that had been starved for

Figure 1. Induction of phage ϕ105 by UV-irradiation

An overnight culture of Bacillus subtilis 168 thy⁻(ϕ105) was diluted 100-fold and grown to exponential phase. This culture was filtered and resuspended in CH medium plus 10 μg/ml thymidine. After growing for 60 minutes, a portion of the culture was taken out and UV-irradiated for 60 seconds. At intervals, viable cells and infectious centers were assayed on NBS agar plates.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10^8
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UV-irradiated infectious centers
Control viable cells
Control infectious centers
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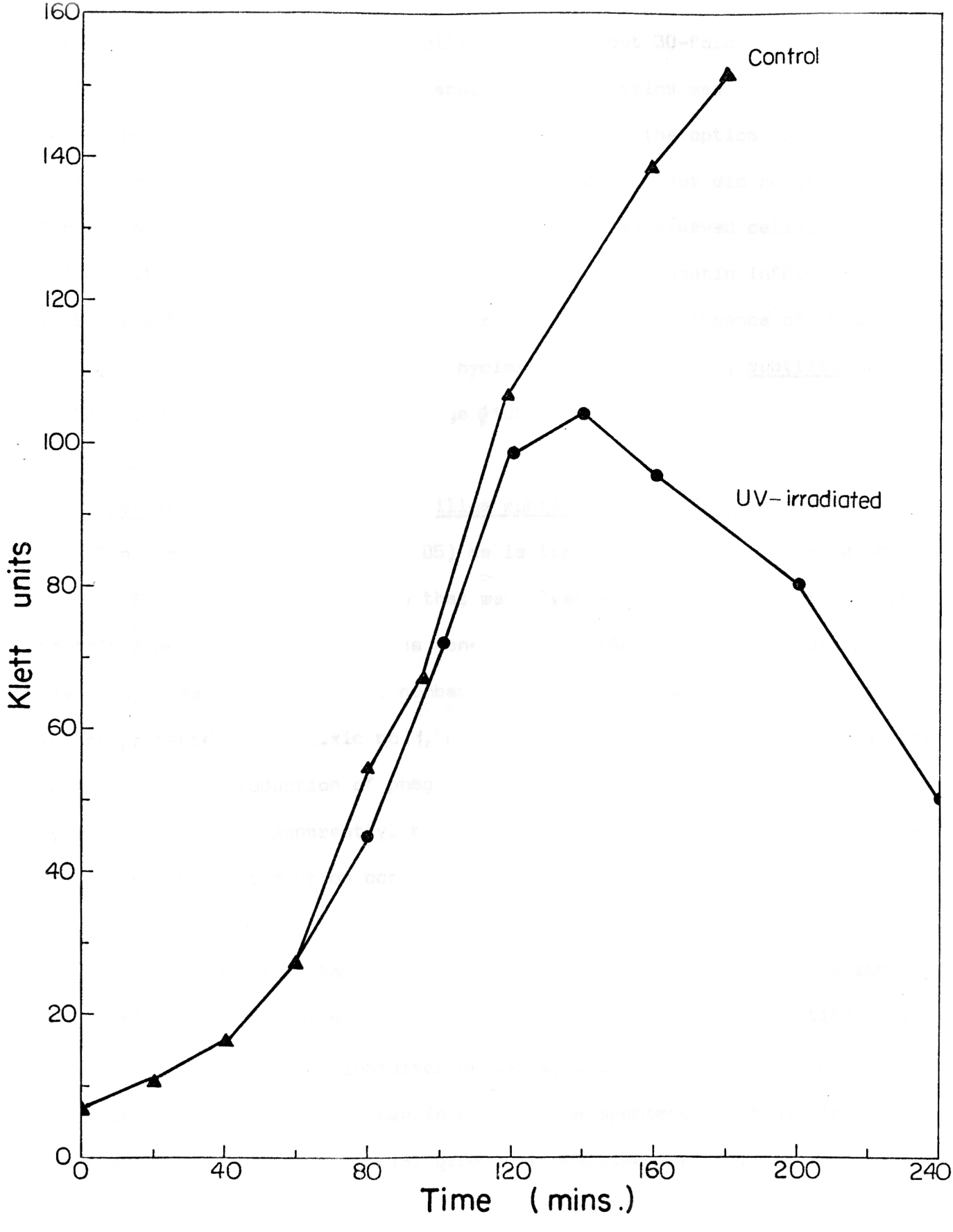
Time (mins.)

Figure 2. The optical density of the cultures described in Fig. 1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240
Time (mins.)

120 minutes, there was a burst of phage production. The number of phage produced was approximate 10 per cell. This is about 30-fold more than that of in thymineless culture. Figure 4 shows that if thymine was not added to thymineless culture, there was no cell lysis, i. e. the optical density of starved culture increased 3-fold and then leveled off but did no decrease. Forty minutes after the addition of thymine to these starved cells, the cell lysed. It is clear that phage ϕ105 does not contain genetic information that will allow the growth of thymine-requiring cells in the absence of thymine. The results also show clearly that thymine deprivation of B. subtilis 168 thy$^-$ (ϕ105) causes the induction of phage ϕ105.

C. Effect of nalidixic acid on Bacillus subtilis 168 thy$^-$(ϕ105):

The survival of 168 thy$^-$(ϕ105) cells treated with nalidixic acid is shown in Fig. 5. These 168 thy$^-$ cells that were lysogenized with ϕ105 were killed by nalidixic acid in the same way as non-lysogenic 168 thy$^-$ cells as described by Ramareddy & Reiter(1969). The number of viable cells decreased exponentially in the presence of nalidixic acid, until at 180 minute 1% of the cells survived. The spontaneous production of phage during this 180 minutes was also inhibited by nalidixic acid. Apparently, as long as nalidixic acid was present in the medium, no phage production occurred.

We have shown that both UV-irradiation and thymine starvation, which could interrupt the synthesis of DNA, could induce prophage production. Since nalidixic acid is also an inhibitor of DNA synthesis, its ability to induce prophage was also tested. As shown in Fig. 5, the spontaneous production of phage parallelled the rate of bacterial growth as described before. In nalidixic acid-treated cells, both cell growth and the spontaneous production of phage

Figure 3. Induction of Bacillus subtilis 168 thy⁻ (ϕ105) by thymine starvation

An overnight culture of B. subtilis 168 thy⁻ (ϕ105) was diluted and grown to logarithmic phase in CH medium plus 10 µg/ml thymidine. These cells were then washed and resuspended in the same medium thymidine. The culture was divided into two portions. 10 µg/ml thymidine was added to one portion(control). Another portion was starved for thymidine. After 120 minutes, 10µg/ml thymidine was added to a portion of the starved cells. At intervals, the culture was assayed for viable cells and infectious centers.

Curve A. Control viable cells
Curve B. Infectious centers in thymine-starved culture
Curve C. Thymineless death
Curve D. Infectious centers in thymine-starved culture after addition of thymidine at 120 minutes of starvation.

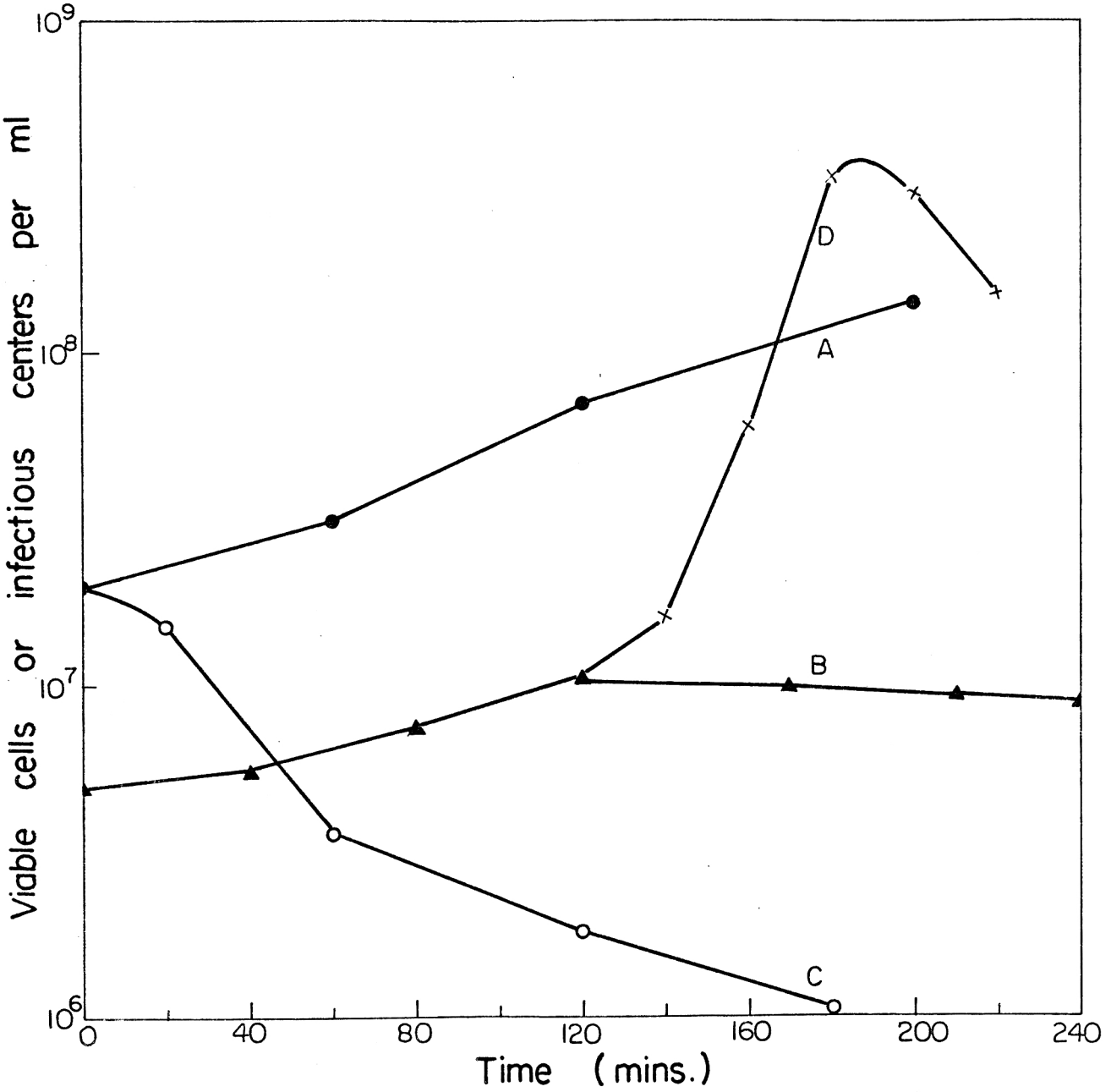
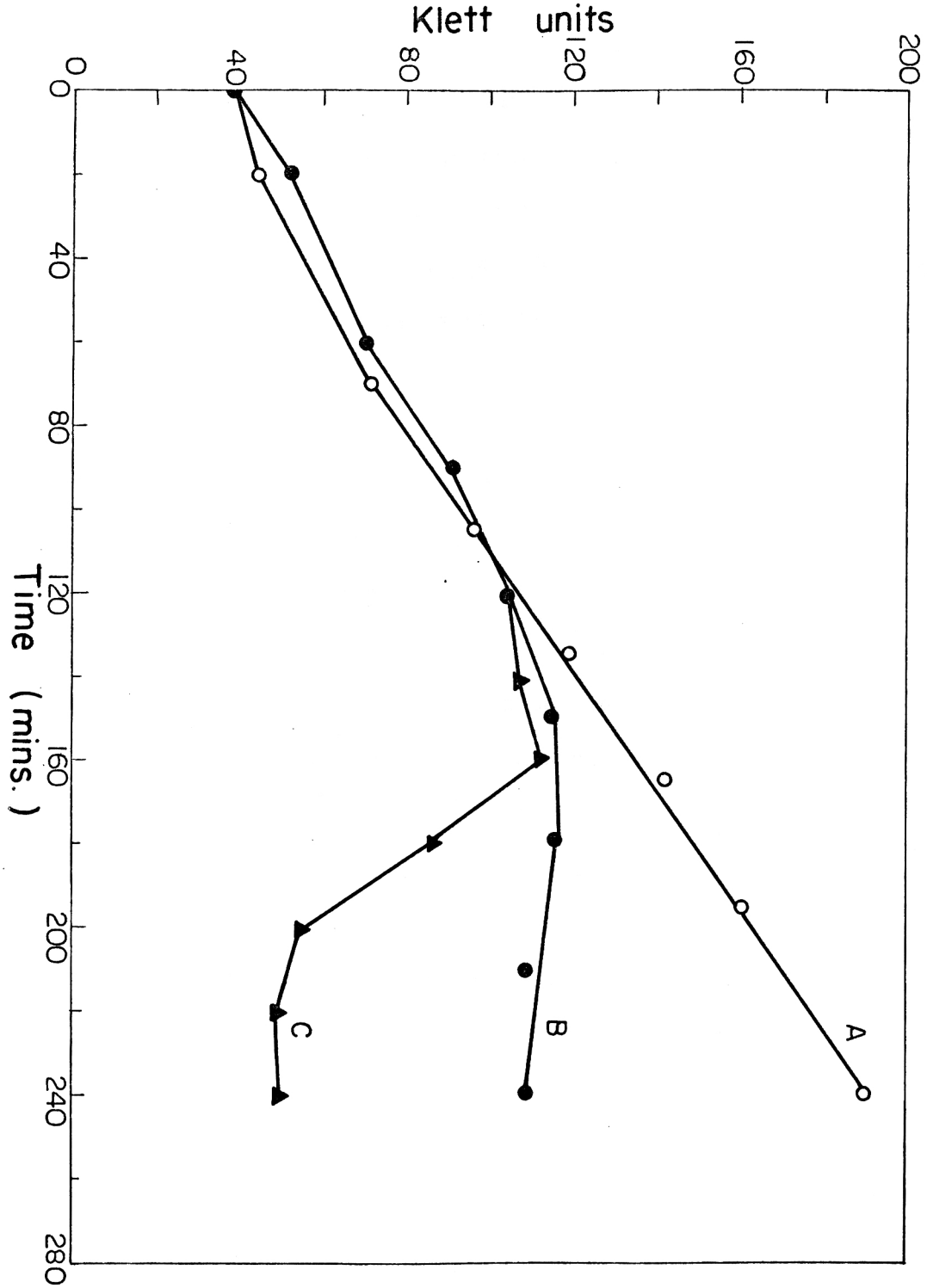

Viable cells or infectious centers per ml
10^9
10^8
10^7
10^6
0
40
80
120
160
200
240
Time (mins.)
A
B
C
D

Figure 4. The optical density of the cultures described in Fig. 3

Curve A. Optical density of control culture
Curve B. Optical density of thymine-starved culture
Curve C. Optical density of thymine-starved culture after addition of thymidine at 120 minutes starvation


Klett units
0
40
80
120
160
200
Time (mins.)
0
40
80
120
160
200
240
280
A
B
C

were inhibited. However, after removal of nalidixic acid from the culture that had been treated with nalidixic acid for 80 minutes, ther was a burst of phage production, about 6 phages per cell.

The burst of phage induction in thymineless induction and in the nalidixic treatment indicated that this treatment caused the first step of induction which predisposed that phage DNA to vegetative replication, as described by Melechen et al.(1962) in E. coli B3(P1b). However, the vegetatives replication was inhibited in the absence of thymine or as long as nalidixic acid was present in the medium.

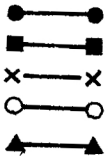
The relation between the durtion of nalidixic acid treatment and prophage induction was examined by experiments, described in Fig. 7, in which nalidixic acid was removed from a culture that had been treated with nalidixic acid for various length of time. It is clear that phage induction occurs after removal of nalidixic acid from 20, 40 and 60 minute of nalidixic acid-treated cells. It is concluded that there is no correction between the time of nalidixic acid treatment and phage induction in the range of 20-80 minutes. The only difference is that there is a longer latent period in the 20 minute nalidixic acid-treated culture than those of 40 and 60 minute. As compare to UV-irradiated induction, it seems that both have the same pattern of induction, except for two points. One is the different latent period and the other is the different latent period with different number of phage produced.

D. Action of nalidixic acid on phage induction after UV-irradiation:

It has been suggested that prophage induction occurs in two steps. The first was the release of the prophage genome from the repressed, integrated

Figure 5. Induction of Bacillus subtilis 168 thy⁻(ϕ105) by nalidixic acid

Exponentially growing cells were filtered and resuspended in CH medium plus 10 μg/ml thymidine. The cells were divided into two portions. One portion continued growing as a control, another portion was treated with 40 μg/ml nalidixic acid. After 80 minute incubation, samples were taken from the treated culture and diluted 100-fold. At intervals, viable cells and infectious centers were assayed.



Control viable cells
Nalidixic acid-treated viable cells
Control infectious centers
Nalidixic acid-treated infectious centers
Infectious centers of nalidixic acid-treated for 80 minutes and diluted 100-fold

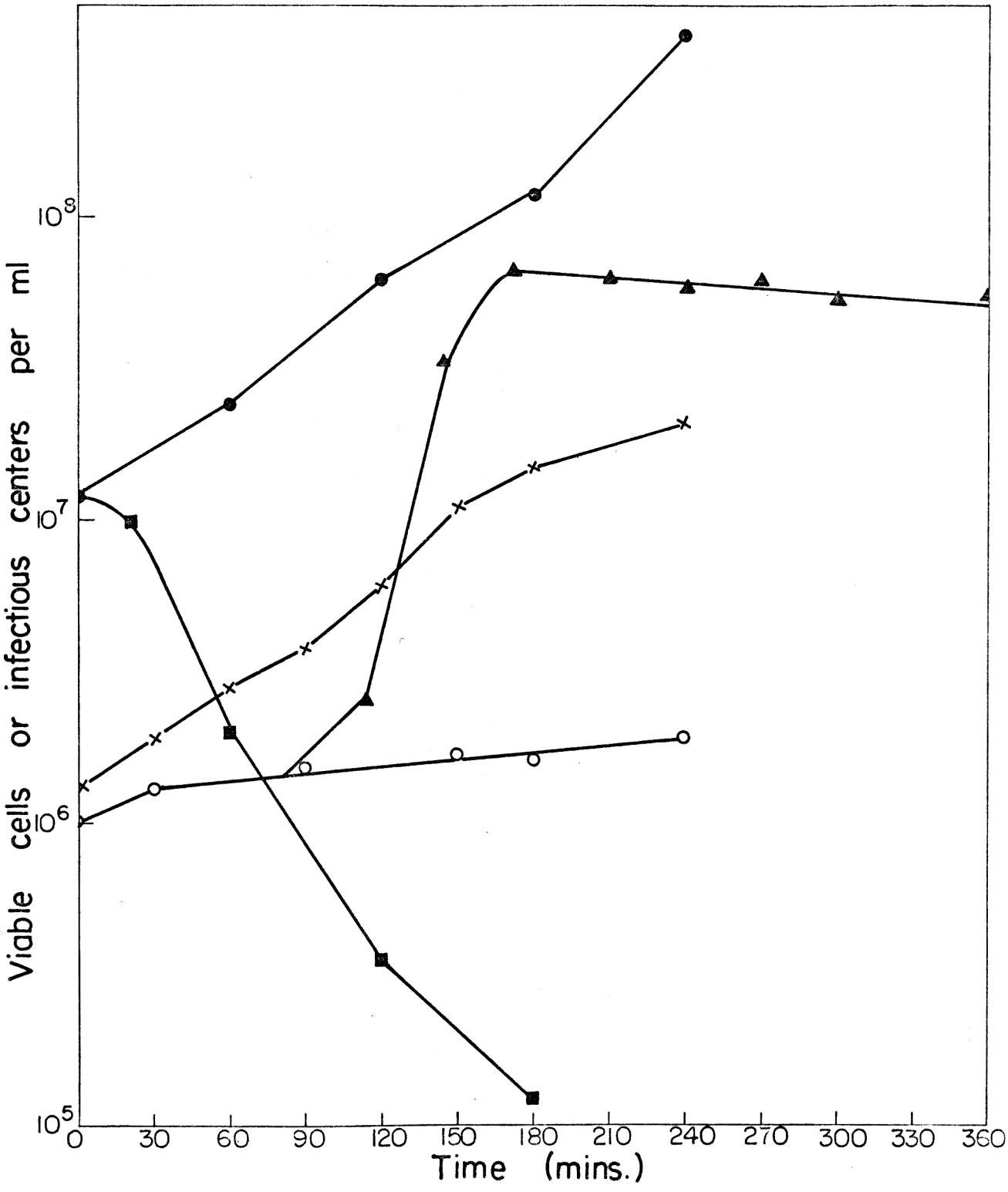
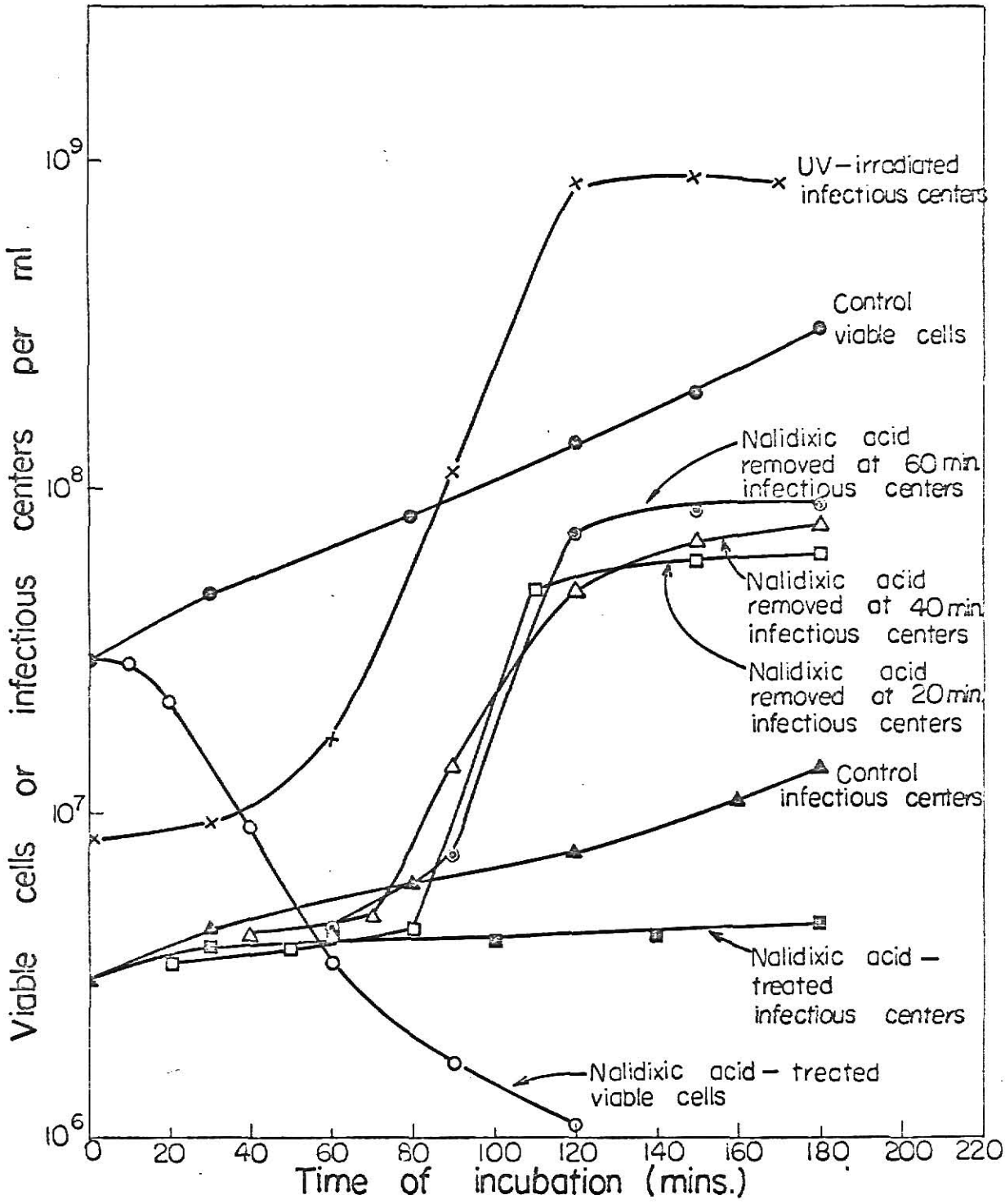

Viable cells or infectious centers per ml
10^8
10^7
10^6
10^5
0
30
60
90
120
150
180
210
240
270
300
330
360
Time (mins.)

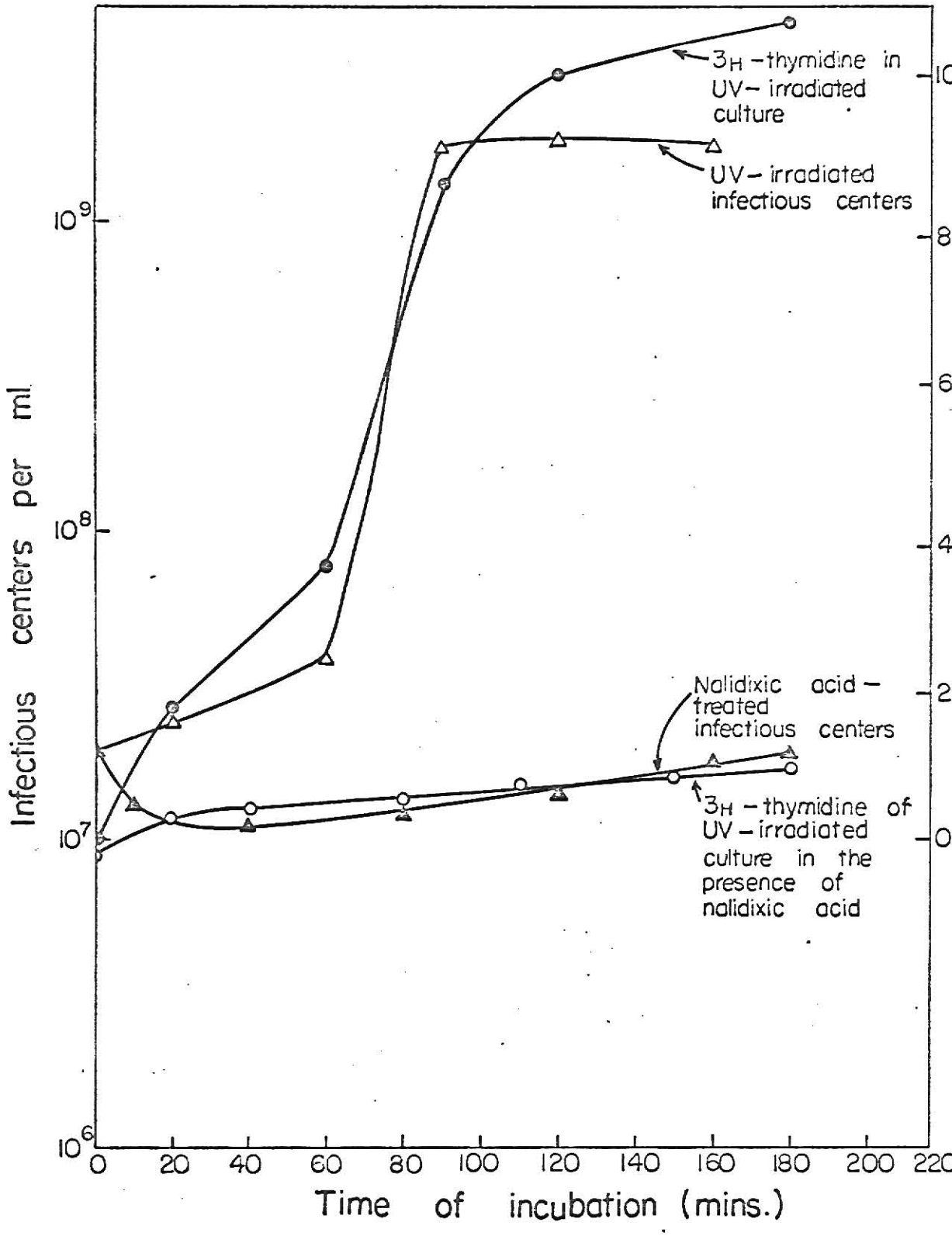
Figure 6. Relation between induction and the duration of nalidixic acid treatment. Exponentially growing 168 thy$^-$(ϕ105) cells were filtered and resuspended in CH medium plus 10 μg/ml thymidine. The cells were divided into three portions. One portion continued growing, another portion was UV-irradiated for 60 seconds(25 ergs/mm^2/sec), and the third portion was treated with 40 μg/ml nalidixic acid. At time 20, 40 and 60 minutes, nalidixic acid treatment was ended by diluting a sample of the cultures 100-fold into the same medium. At intervals, viable cells and infectious centers were assayed on NBS plates.


Viable cells or infectious centers per ml
10^9
10^8
10^7
10^6
0
20
40
60
80
100
120
140
160
180
200
220
Time of incubation (mins.)
UV-irradiated infectious centers
Control viable cells
Nalidixic acid removed at 60 min. infectious centers
Nalidixic acid removed at 40 min. infectious centers
Nalidixic acid removed at 20 min. infectious centers
Control infectious centers
Nalidixic acid-treated infectious centers
Nalidixic acid-treated viable cells

state. The second was the replication of the derepressed phage genome. It has been postulated that UV-irradiation caused the degradation of cell DNA and that the degradation products antagonized the repressor and led to the derepression of the prophage genome. Since nalidixic acid has been found to inhibit the spontaneous production of prophage, the following experiments were made to determine the effects of nalidixic acid on prophage induction, i. e. whether it inhibited the derepression of the prophage genome or the replication of the phage genome. Logarithmically growing B. subtilis 168 thy⁻ (ϕ105) cells were irradiated by UV light for 60 seconds. 2 μC/ml ^{3}H-thymidine was then added to the irradiated culture. The culture was divided into two portions. One portion served as a control. The other portion was treated with 40 μg/ml nalidixic acid. At intervals, viable cells, infectious centers, and the amount of ^{3}H-thymidine incorporation into DNA were assayed. The results were shown in Fig. 7. Where nalidixic acid was added to the cells immediately after UV-irradiation, DNA synthesis was arrested after 10 minutes. Nalidixic acid also inhibited vegetative development of phage ϕ105 after UV-irradiation. It seems, therefore, that DNA synthesis is necessary for phage ϕ105 to be produced.

In a second series of experiments, the cells were first treated with 40 μg/ml nalidixic acid for 20 minutes and UV-irradiated in the continued presence of nalidixic acid. Figure 8 shows that there was still a three fold increase of infectious centers immediately after UV-irradiation even in the presence of nalidixic acid. However, there was no further phage production in the presence of nalidixic acid after UV-irradiation. Figure 9 also shows that there was no cell lysis in the presence of nalidixic acid after UV-irradiation.

Figure 7. The effect of nalidixic acid on phage production in UV irradiated Bacillus subtilis 168 thy⁻(Ø105). An overnight culture of 168 thy⁻(ϕ105) cells was diluted and grown to logarithmic phase in CH medium plus 10 μg/ml thymidine. The culture was then UV-irradiated for 60 seconds. 2 μC/ml of ^{3}H- thymidine was added. The culture was divided into two portions. One portion served as a control. The other portion was treated with 40 μg/ml nalidixic acid. At intervals, viable cells, infectious centers were assayed and ^{3}H-thymidine incorporation was also measured.


^3H – thymidine in UV – irradiated culture
UV – irradiated infectious centers
Nalidixic acid – treated infectious centers
^3H – thymidine of UV – irradiated culture in the presence of nalidixic acid
Infectious centers per ml
^3H – incorporation, cpm (X 100)
Time of incubation (mins.)
10^9
10^8
10^7
10^6
0
20
40
60
80
100
120
140
160
180
200
220
10
8
6
4
2
0

Figures 8. An overnight culture of Bacillus subtilis 168 thy⁻(ϕ105) was diluted and grown to logarithmic phase in CH medium plus 10 μg/ml thymidine. The culture was then divided into two portions. One portion served as a control. The other portion was treated with 40 μg/ml nalidixic acid. After 20 minutes, samples from both cultures were taken and UV-irradiated for 60 seconds. At intervals, viable cells and infectious centers were assayed. Optical density was also measured.

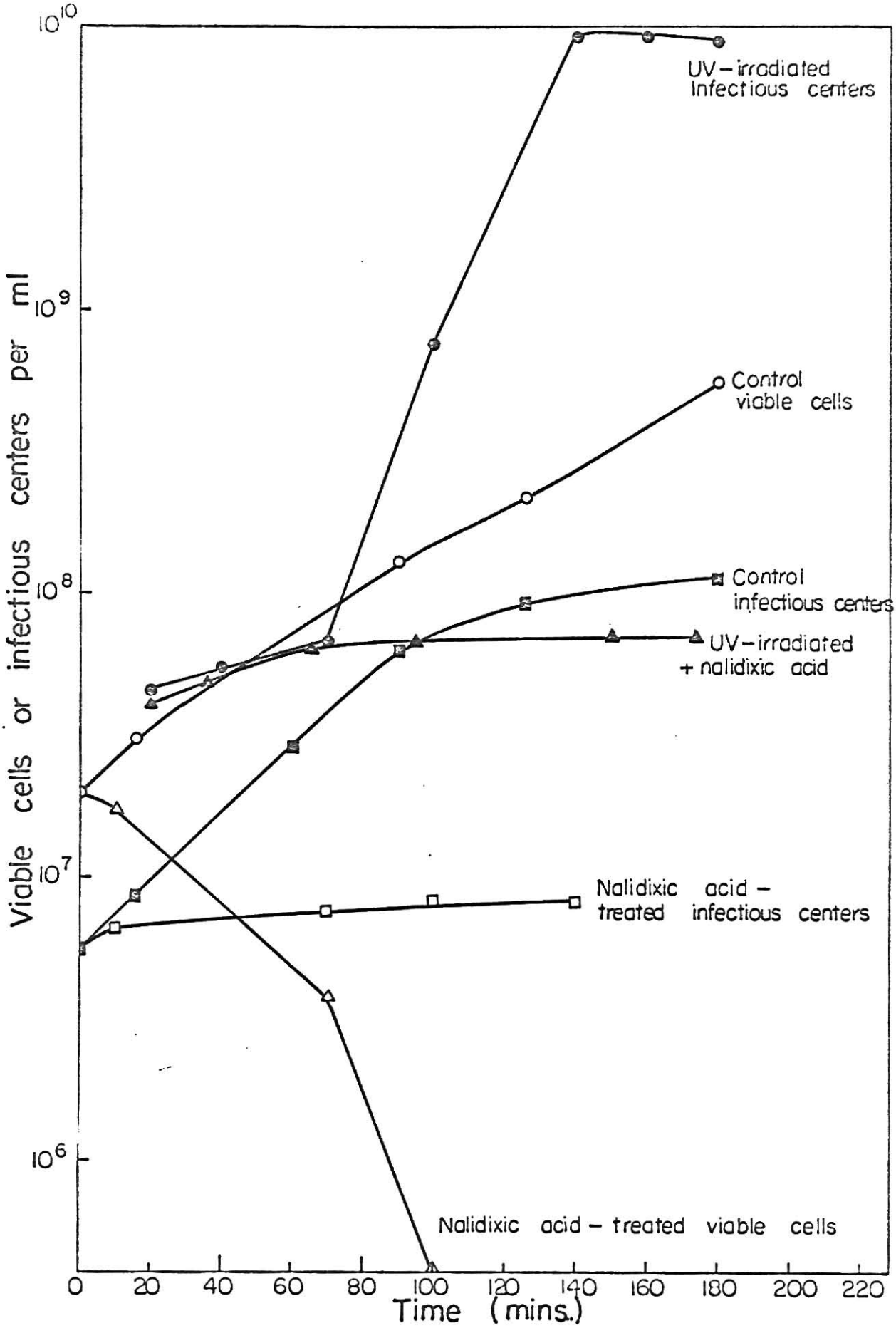
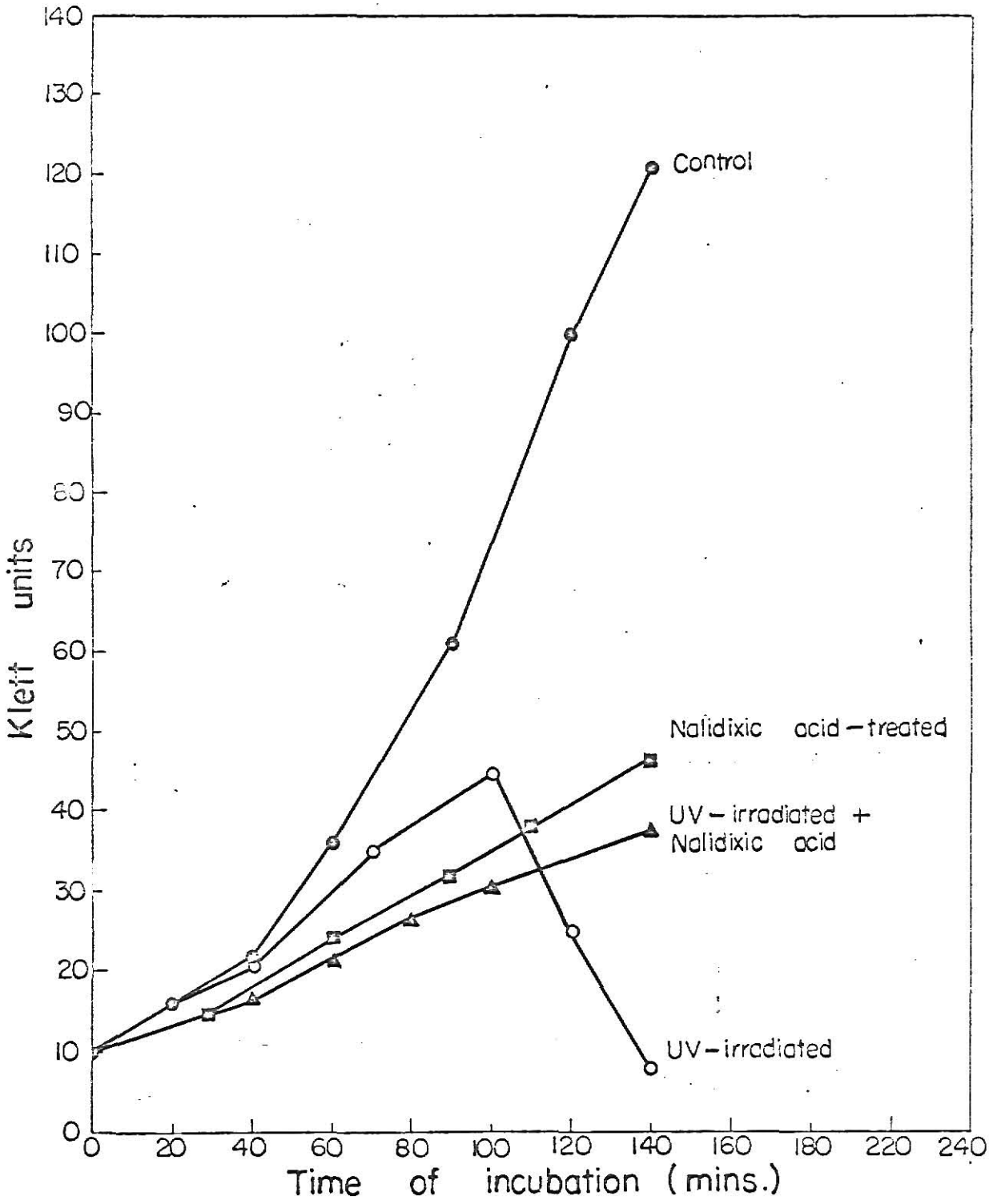

Viable cells or infectious centers per ml
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
UV-irradiated Infectious centers
Control viable cells
Control infectious centers
UV-irradiated + nalidixic acid
Nalidixic acid - treated infectious centers
Nalidixic acid - treated viable cells
0
20
40
60
80
100
120
140
160
180
200
220
Time (mins.)

Figure 9. The optical density of the cultures described in Fig. 8.


Klett units
Time of incubation (mins.)
Control
Nalidixic acid – treated
UV – irradiated + Nalidixic acid
UV – irradiated
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
0
20
40
60
80
100
120
140
160
180
220
240

It seems from these experiments that nalidixic acid did not inhibit the derepression of phage genome; rather, it inhibited the replication of derepressed phage genome. These results are consistent with the hypothesis that nalidixic acid treatment caused the first step of induction, i. e. the conversion of prophage DNA from the integrated, repressed state to the excised, vegetative state. Upon the removal of nalidixic acid from the treated cells, phage DNA began to replicate.

UV-irradiation causes the degradation of cell DNA at random points along the chromosome. It has been hypothesized that this degradation involves in the inactivation of repressor and leads to the derepression of phage genome, following by excision and replication of phage genome. Recently, it has been reported from this laboratory that nalidixic acid, thymine starvation and UV-irradiation cause degradation of DNA behind the replicating point. It is tempting to spaculate that the replicating point on the cell chromosome upon reaching prophage integration site may be damaged with nalidixic acid or thymine starvation and resulting in prophage induction. i. e. whether phage induction occurs when the degradation caused by nalidixic acid happened to be near the site of prophage integration. In order to test this possibility, we must get the synchronized cells that are replicating their chromosome near the prophage integration site. Bacillus subtilis 168 thy$^-$ind$^-$ts-134 was selected to synchronize the cells, since this temperature sensitive mutant is thought of a DNA-initiator mutant (Mendelson & Gross, 1967). They showed that DNA synthesis in this mutant was arrested after 30 minutes of incubation at 45°C, and the initiation of new round of DNA synthesis was inhibited. As a preliminary study, some characteristics of the effects of high temperature on this mutant were examined.

E. DNA synthesis and phage production in Bacillus subtilis 168 thy⁻ind⁻ts-134 (ϕ105) at high temperature.

DNA synthesis was examined by ^{3}H-thymidine incorporation in this temperature sentive mutant. Figure 10 shows that after transferring the cells to 45°C, the rate of DNA synthesis increased about two fold as in 30°C and then arrested. However, DNA synthesis was resumed if the culture was transferred back to 30°C. It is clear from Figure 10 that DNA syntnesis was arrested after 45 minute incuabation of this ts mutant at 45°C.

In another series of experiments, phage production of this ts mutant at 45°C was also examined. An overnight culture was diluted and grown to logarithmic phase at 30°C in CH medium plus 10 μg/ml thymidine and 40 μg/ml tryptophan. The culture was transferred to 45°C and grown for 45 minutes to arrest DNA synthesis after the completion of all replicating chromosome. The cells were then divided into two portions. One portion continued growing at 45°C and the other portion was transferred to 30°C. At intervals, viable cells and infectious centers were assayed. Figure 11 shows that at 45°C phage production increased about two fold during 45 minute incubation and then was inhibited. However, after transferring back to 30°C, phage production and viable cells increased. So the spontaneous production of phage in this ts mutant was temperature sensitive.

F. Inhibition of phage induction after UV irradiation at 45°C in Bacillus subtilis 168 thy⁻ind⁻ ts-134 (ϕ105).

As was shown above, DNA synthesis was necessary for phage ϕ105 to be produced. This finding was examined in the ts mutant at 45°C in which DNA synthesis was arrested. An overnight culture of this temperature sensitive

Figure 10. DNA synthesis in Bacillus subtilis 168 thy⁻ind⁻ ts-134 (ϕ105) at 45°C.

Cells were grown overnight at 30°C in CH medium plus 10 µg/ml thymidine and 40 µg/ml tryptophan. The culture was then diluted and grown to logarithmic phase in the same medium at 30°C. To this culture, 1 µC/ml ^{3}H-thymidine was added. After growing for 60 minutes for 30°C, the cells were transferred to 45°C. After 20, 70, and 120 minutes of growth at 45°C, a portion of the cultures are taken and transferred back to 30°C. At intervals, ^{3}H-thymidine in these cultures was measured for DNA synthesis.

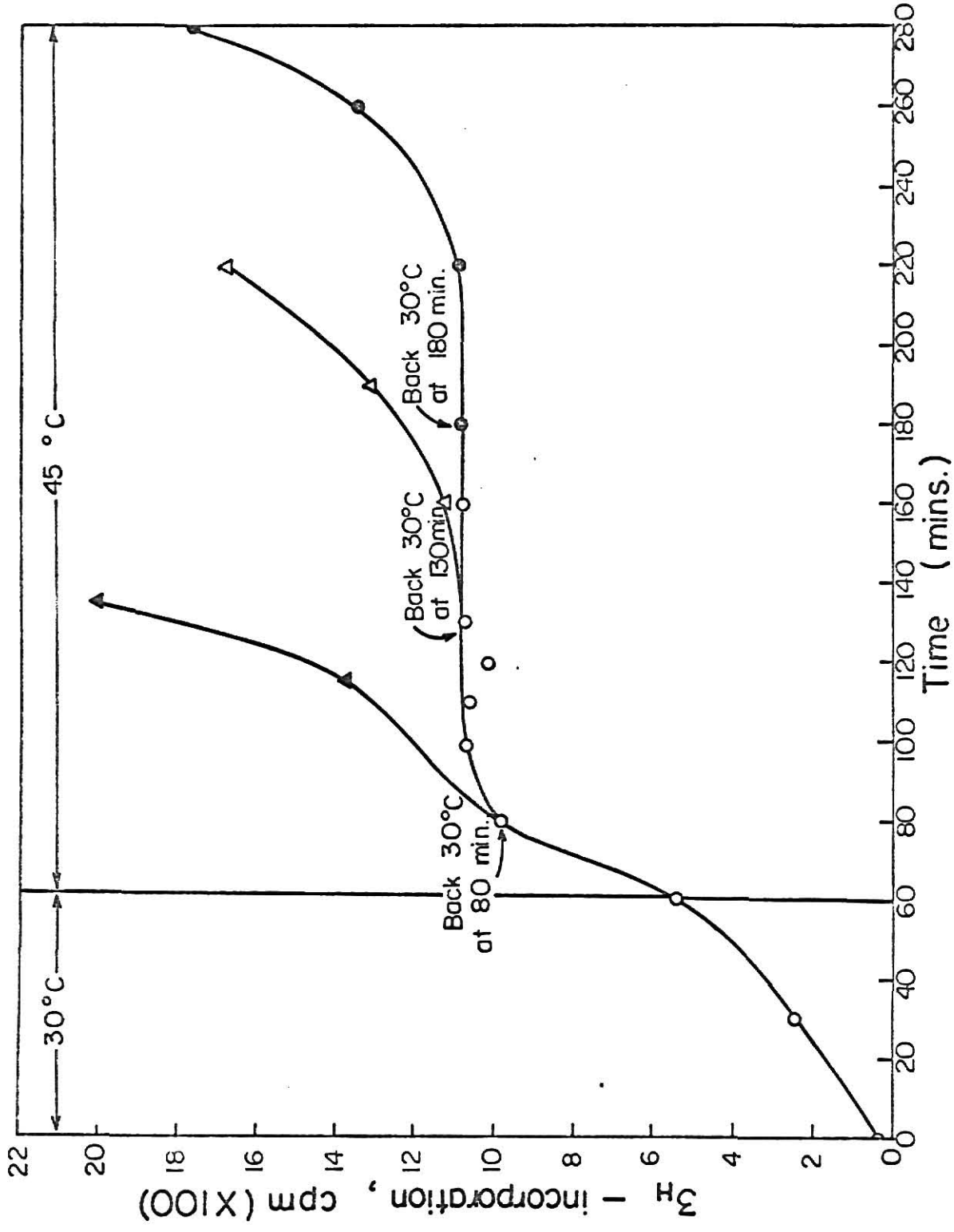
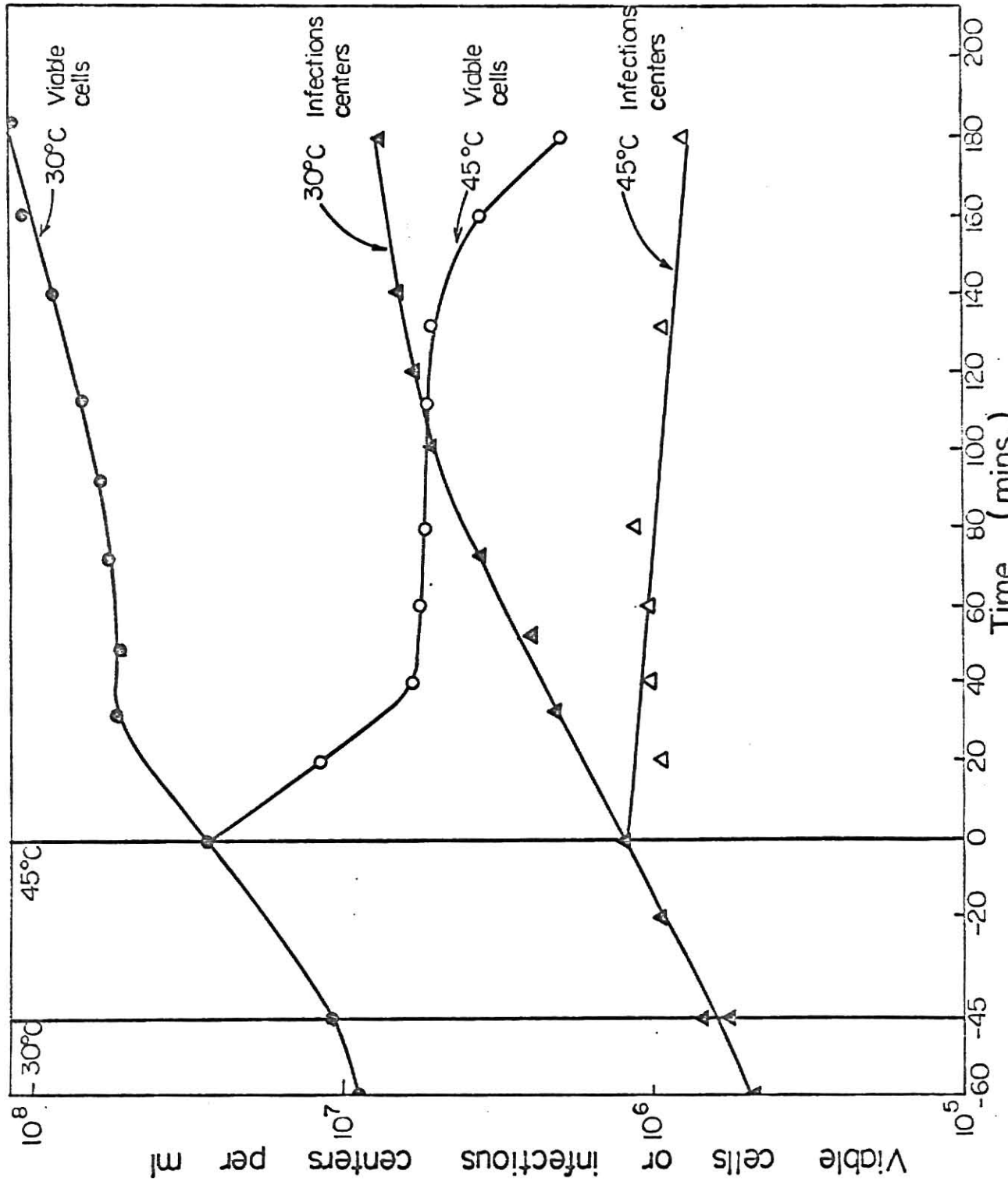

^3H — incorporation , cpm (X100)
Time (mins.)
30°C
45 °C
Back 30°C at 80 min.
Back 30°C at 130min.
Back 30°C at 180 min.

Figure 11. Inhition of phage production in Bacillis subtilis 168 thy⁻ ind⁻ ts-134(ϕ105) at 45°C.

Lysogenic cells of the ts mutant were grown overnight at 30°C in CH medium plus 10 µg/ml thymidine and 40 µg/ml tryptophan. The culture was diluted and grown to logarithmic phase at 30°C in the same medium. The cells were then transferred to 45°C for 45 minutes and divided into two portions. One portion continued growing at 45°C and the other portion was transferred to 30°C. At intervals, viable cells and infectious centers were assayed.


Viable cells or infectious centers per ml
10^8
10^7
10^6
10^5
30°C
45°C
Viable cells
30°C
Infections centers
45°C
Viable cells
45°C
Infections centers
-60
-45
-20
0
20
40
60
80
100
120
140
160
180
200
Time (mins.)

mutant was diluted and grown at 30°C to logarithmic phase in CH medium plus 10 μg/ml thymidine and 40 μg/ml thymidine and 40 μg/ml tryptophan. This culture was transferred to 45°C for 45 minutes at which time DNA synthesis was arrested. This culture was then UV-irradiated for 60 seconds and divided into two portions. One portion continued growing at 45°C, and the other was transferred to 30°C. At intervals, infectious centers were assayed. Figure 12 shows that after UV-irradiation at 45°C, there was a three fold increase in infectious centers. However, phage production was inhibited by continuous incubation at 45°C after UV-irradiation. After UV-irradiation there was a typical phage induction, when the cells were incubated at 30°C. This is similar to the observations of the earlier experiments with nalidixic acid in which it was noted that DNA synthesis was necessary for phage ϕ105 produced.

C. Relation between phage induction and the replicating point.

Rutberg (1969) showed that phage ϕ105 integrated linearly into the Bacillus subtilis 168 chromosome between the phe-1 and ilv-A1 markers upon lysogenization. These markers are located about 80% from the origin of the B. subtilis chromosome. Since the generation time of this ts mutant in this medium is about 60 minutes, so the logarithmically growing cells, which have been transferred to 45°C for 45 minutes, were grown at 30°C for 45 minutes. At that time the cells are supposed to replicate near the prophage integration site. Figure 13 shows there was phage induction by nalidixic acid after 45 minute growth at 30°C. However, there were phage inductions in both 0 and 80 minute growth at 30°C in which the cells are not supposed to replicate thier chromosome near the prophage integration site. It was then concluded that there is no relation between the position of replicating point and phage induction after nalidixic acid treatment.

Figure 12. Inhibition of phage production after UV-irradiation at 45°C.

An overnight culture of B. subtilis 168 thy⁻ind⁻ts-134(ϕ105) was diluted and grown to the early logarithmic phase in CH medium plus 10 µg/ml thymidine and 40 µg/ml tryptophan. The cells were incubated at 45°C for 45 minutes and then UV-irradiated for 60 seconds. The cells were divided into two portions. One portion continued growing at 45°C and the other portion was transferred back to 30°C. At intervals, infectious centers were assayed on NBS plates.

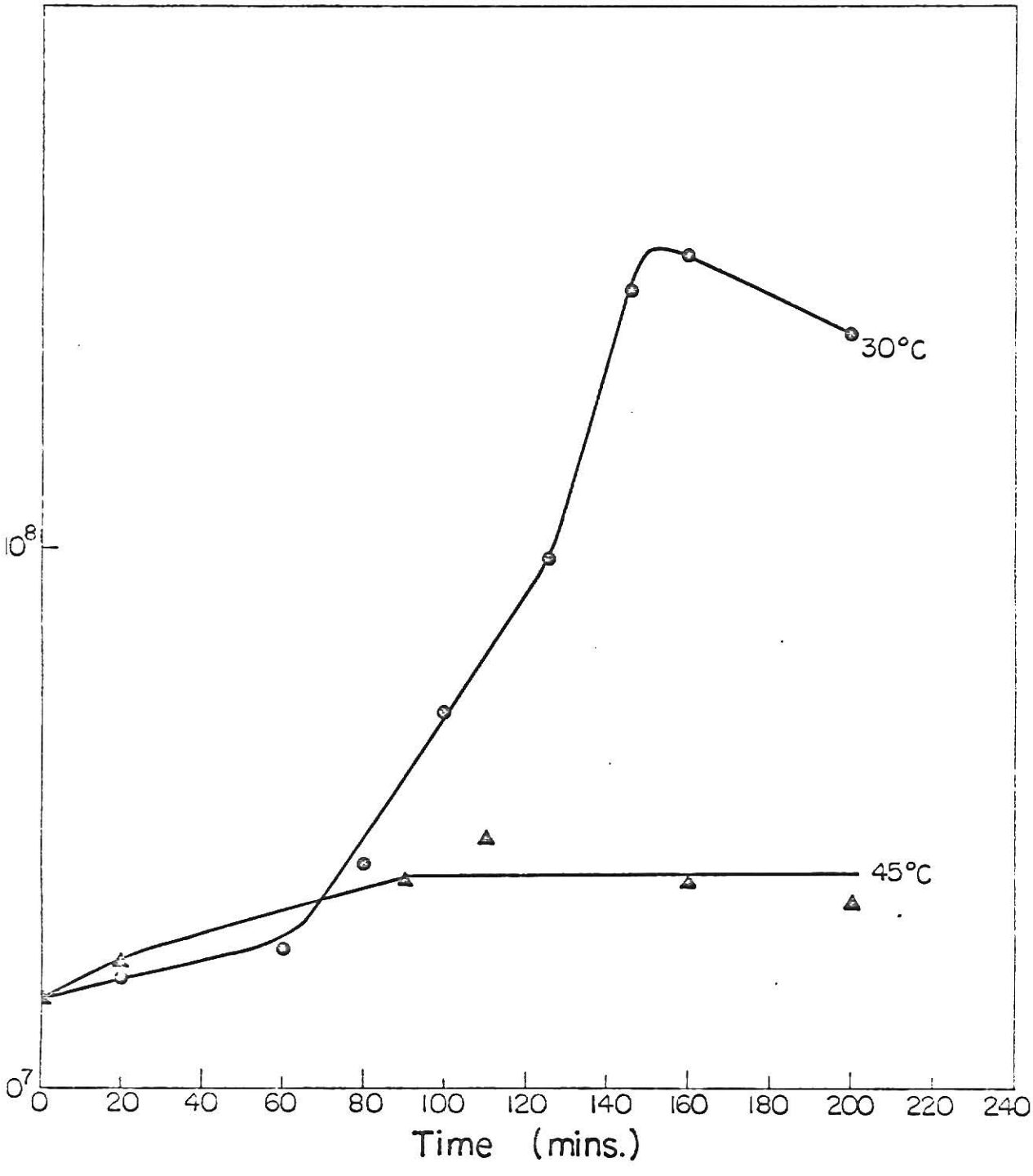
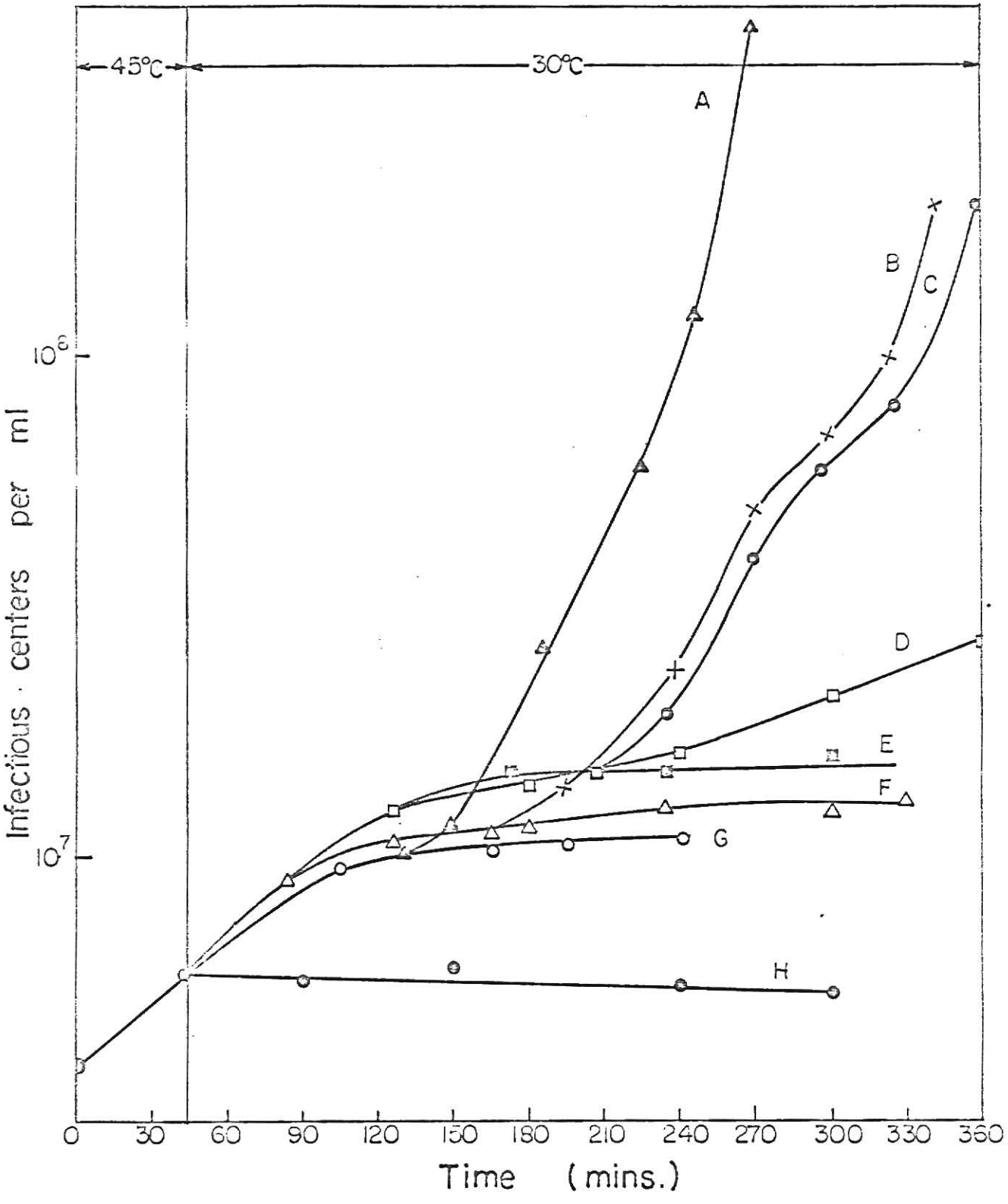

30°C
45°C
10^8
0^7
0
20
40
60
80
100
120
140
160
180
200
220
240
Time (mins.)

Figure 13. Relation between the replicating point and prophage induction.

Logarithmically growing culture of B. subtilis 168 thy⁻ind⁻ts-134(ϕ105) in CH medium plus 10 μg/ml thymidine and 40 μg/ml tryptophan was transferred from 30°C to 45°C and incubated for 45 minutes. The cells were then transferred back to 30°C. One portion was immediately treated with nalidixic acid. The other portions were grown at 30°C for 45 minutes and 80 minutes respectively, and then both treated with nalidixic acid. All these three portion were treated with 40 μg/ml nalidixic acid for 80 minutes and then diluted 100-fold. At intervals, infectious centers were assayed.

A. Nalidixic acid treated for 80 minutes and then diluted
B. Grown for 45 minutes and then treated with nalidixic acid for 80 minutes and diluted
C. Grown for 80 minutes and then treated with nalidixic acid for 80 minutes and diluted
D. Control
E. Grown for 80 minutes and then treated with nalidixic acid
F. Grown for 45 minutes and then treated with nalidixic acid
G. Nalidixic acid-treated
H. 45°C


45°C
30°C
A
B
C
D
E
F
G
H
10^8
10^7
Infectious centers per ml
0
30
60
90
120
150
180
210
240
270
300
330
360
Time (mins.)

DISCUSSION

The results in this thesis indicate that UV-irradiation induces prophage. Immediately after UV-irradiation, there is a threo-fold increase in the number of infectious centers in the culture. This increase is followed by a latent period of 20 minutes during which time no increase in infectious centers occurs. After this 20 minutes latent period there is an exponential increase in the number of infectious centers until a cycle of phage production wuth an average burst size of 60 phages per cell is completed 100 minutes after the time of irradiation.

The experiments also show that thymine starvation causes prophage induction. During first 120 minutes of thymine starvation, there is less than two-fold increase in the number of infectious centers in the cultures. This number never exceeds the number of cells initially present at the start of starvation. Only upon the readdition of thymine to the culture, does the number of infectious centers in the culture increase. The burst size in thymine induced cells is always approximate 10 phages per cell less than the number produced in UV-induced cultures.

The results show a third agent, nalidixic acid, also induces prophage . As in the case of thymine starvation, there is a two-fold increase of infectious centers in a culture treated with nalidixic acid for 240 minutes. Phage production is initiated upon removal of nalidixic acid from the culture. The number of phage produced in the nalidixic acid induced culture is approximate 6 phages per cell. I have also found that 20 minutes of treatment with nalidixic acid is as effective in inducing prophage as 40 or 60 minutes of treatment.

The three-fold increase in infectious centers that occurs immediately after UV-irradiation is equal to the number of infectious centers expected if each lysogenic cell yields a plaque upon plating. This complete induction does no occur in the thymine starved and nalidixic acid-treated cultures in which even after four hours of incubation, the number of infectious centers never exceeds twice the initial number in the culture.

As indicated in the Introduction section, induction may be divided into two steps. The first is the alteration of the repressed, integrated prophage to the excised, vegetative state. The second is the actual replication of the derepressed, vegetative phage. The data in this thesis shows that the first step of induction, i. e. derepression can occur in the absence of DNA synthesis. As shown in Fig. 8 and Fig. 12, UV-irradiation causes a three-fold increase of the number of infectious centers even in the presence of nalidixic acid or at a temperature at which DNA synthesis is inhibited. However, further production of phage is inhibited by the continued treatment with nalidixic acid or high temperature. This suggests that the second step in induction requires DNA synthesis.

The way that inducing agents bring about the first step of induction is not as yet clear. However, all inducing agents appear to have common property of inhibiting, specifically, DNA synthesis. Three hypotheses have been proposed to explain the mechanism of the first step of induction. On is the precursor hypothesis. That is , that in the absence of DNA synthesis, DNA precursors accumulate, and that upon reaching a sufficient concentration, than these precursors will antagonize and inactivate the repressor that maintains the

lysogenic state. A second hypothesis is the the inducing agents cause the degradation of DNA and the products of degradation antagonize the repressor, thus resulting the prophage diverted to vegetative growth. The third hypothesis is that the replicating point in the cell chromosome at the site of the integrated prophage might be damaged by the inducing agents. Consequently, the degradation of DNA at the growing point that is caused by nalidixic acid, thymine starvation and UV-irradiation would result in the excision of prophage located near the replicating point. The results in this thesis indicate that the third hypothesis may not be correct.

The experiments(Fig. 13) in which the temperature sensitive, DNA-initiator mutant was used to align the DNA replicating point near the chromosome origin (12 to 1 o'clock position), shows that nalidixic acid causes the induction of prophage integrated at the 10 o'clock position in the chromosome. Thus, phage induction does not appear to be the consequence of degradation of the DNA near the site of prophage integration. Another observation, that the arrest of DNA synthesis in the temperature sensitive, DNA-initiator mutant does not lead to the induction of prophage, indicate that induction is also no caused by the accumulation of DNA precurssors. Such precurssors would be expected to have accumulated in the temperature arrested cells. Thus the second hypothesis, that induction is the function of DNA degradation remain as most likely to be correct. This conclusion is supported by a recent observation, not reported in this thesis, that cell is unable to excise UV-induced pyrimidine dimers from its DNA, and consequently unable to degrade moch of its DNA also non-inducible by UV-irradiation.

SUMMARY

1. UV-irradiation, thymine starvation, and nalidixic acid treatment, induced phage ϕ105 in Bacillus subtilis.

2. Production of phage ϕ105 in Bacillus subtilis was inhibited by nalidixic acid as long as nalidixic acid was present.

3. 20 minutes of treatment with nalidixic acid was a efficient in inducing prophage as 40 or 60 minutes of treatment.

4. A temperature sensitive, DNA-initiator mutant was used to arrest and synchronize DNA synthesis. The efficiency of induction in these synchronized cells was independent of the positions of the replicating points at the time of induction.

ACKNOWLEDGEMENTS

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INDUCTION OF PROPHAGE IN *BACILLUS SUBTILIS* 168(ϕ105)

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AN ABSTRACT OF A MASTER'S THESIS

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Induction of prophage in Bacillus subtilis 168(ϕ105) was studied. It was found that UV-irradiation, thymine starvation, and nalidixic acid treatment induced phage ϕ105 in this lysogenic strain. It was also found that 20 minutes of treatment with nalidixic acid was as efficient in inducing prophage as 40 or 60 minutes of treatment.

Production of phage ϕ105 in Bacillus subtilis was inhibited by nalidixic acid as long as nalidixic acid was present in the medium.

A temperature sensitive, DNA-initiator mutant was used to arrest and synchronize DNA synthesis. It was found that the efficiency of induction was independent of the positions of the replicating point at the time of induction.