ON THE INDUCTION OF ESCHERICHIA COLI K12 (λ) BY AMINOPTERIN

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There are many similarities between the induction of lysogenic and bacteriocinogenic systems (Jacob and Wollman, 1959); however, not enough is known on the mechanism of induction, to evaluate accurately the analogy between the two phenomena.

It was reported (Ben-Gurion, 1962) that aminopterin can induce bacteriocinogenic bacteria. The present paper describes the induction, by aminopterin, of a lysogenic system under ordinary conditions of growth and during periods of adjustment to growth in a new medium, and the reversal of this induction by thymin.

Cells of E.coli K12 (λ) were grown overnight at 37° in two different liquid media:- in synthetic medium (Na_2HPO_4, 0.7% w/v, KH_2PO_4, 0.3% w/v, NH_4Cl, 0.1% w/v, NaCl, 0.05% w/v, $CaCl_2$ 10^{-4} M, $MgSO_4$ 10^{-3} M, glucose 0.4% w/v, threonine 0.016% w/v, leucine 0.016% w/v and thiamine 5 γ/ml) and in tryptose (Difco tryptose 2% plus thiamine 5 γ/ml). The following morning each culture was diluted into fresh medium and incubated with aeration for another 2 hours, until the density of the bacteria reached 1 to $2x10^8$ cells/ml. The bacteria from either medium were divided into two batches; each batch was washed and resuspended at the same density of cells in two media, synthetic

medium and tryptose, so that there were now four suspensions: 1 - cells grown all the time in synthetic medium; 2 - cells transferred from synthetic medium to tryptose; 3 - cells grown in tryptose only and 4 - cells transferred from tryptose to synthetic medium. From each of the four suspensions samples were taken and treated as follows:- sample (a) was irradiated with ultraviolet light for 60 seconds; conditions of irradiation were as described previously (Ben-Gurion and Hertman 1958); to sample (b) aminopterin (California Corporation for Biochemical Research) at the dose of 200 γ/ml was added; sample (c) was irradiated with ultraviolet light and treated with aminopterin as (b) and sample (d) was the untreated control. The suspensions were shaken in a water bath at 37° for 2½ hours, then diluted (1:100,00) into tryptose. The dilution was necessary to permit the cells which had been induced by aminopterin, to produce mature phages; this would not have been possible if aminopterin had not been diluted out at this stage.

The diluted cells were incubated for another 2½ hours, and the number of the free phages was determined in each sample, after adding chloroform to kill the surviving bacteria.

The results of a typical experiment are summarized in Table 1.

In order to determine whether aminopterin induces the lysogenic bacteria or only increases the burst size, the number of induced bacteria was measured immediately after the cells were incubated 2½ hours with aminopterin. Each sample was then washed three times in cold phosphate buffer to remove the free phage, diluted into 2 ml of top layer agar (Adams, 1950) containing streptomycin resistant indicator

TABLE 1

Phage production by lysogenic bacteria treated with U.V. and aminopterin under conditions of adjustment to growth in new media and with no such adjustment

Cell treated with	Number of phage particles in 1 ml produced by			
	1 - cells in synthetic medium	2 - cells transferred from synthetic medium to tryptose	3 - cells in tryptose	4 - cells transferred from tryptose to synthetic medium
Control	2.7×10^7	3.2×10^7	6.7×10^7	1.6×10^7
U.V.	8×10^8	3.3×10^8	3.1×10^8	5×10^8
Amino-pterin	1.3×10^8	8×10^6	6×10^8	5×10^8
U.V. & amino-pterin	5.1×10^8	1.7×10^8	2.3×10^8	1.6×10^9

bacteria and poured on top of thick agar plates. These plates were incubated at 37° for 2½ hours and then a second top layer agar containing streptomycin was added. The plaques eventually observed on these plates represented the number of induced cells in the sample at the time of plating. The results of a typical experiment are summarized in Table 2.

TABLE 2

Induction of lysogenic bacteria by aminopterin under conditions of adjustment to growth in a new medium and of no such adjustment.

Cell treated with	Number of induced bacteria in 1 ml produced by			
	1 - cells in synthetic medium	2 - cells transferred from synthetic medium to tryptose	3 - cells in tryptose	4 - cells transferred from tryptose to synthetic medium
Control	2.1×10^5	1.7×10^5	4.3×10^5	1.7×10^5
Amino-pterin	3.4×10^6	1×10^5	5×10^6	1×10^7

Aminopterin induces bacteria grown in synthetic medium or in tryptose; bacteria that have been transferred from a poor to a rich medium have lost their "aptitude" (Lwoff, 1953) to be induced by aminopterin.

When thymin was added to the induced cells, aminopterin induction was inhibited in contrast to the results obtained with bacteriocinogenic system (Ben-Gurion, 1962).

These experiments were carried out as those described above and summarized in Table 2, except that every sample was divided into two parts and thymin (100 γ/ml) was added to one of them. These experiments are summarised in Table 3.

TABLE 3

The effect of thymin on aminopterin induction of E.coli K12 (λ)

Cell treated with	Number of induced bacteria in 1 ml produced by			
	1 - cells in synthetic medium	2 - cells transferred from synthetic medium to tryptose	3 - cells in tryptose	4 - cells transferred from tryptose to synthetic medium
Control	1.9×10^5	1.5×10^5	7.3×10^5	1.9×10^5
Aminopterin	2.8×10^6	9×10^4	7.6×10^6	1×10^7
Aminopterin & thymin	1.6×10^5	1×10^5	4.5×10^5	1.8×10^5

The experiments described in this report show that aminopterin can induce E.coli K12 (λ), and that thymin counteracts aminopterin effect.

These observations and others (Ben-Gurion 1962; Melechen and Skaar 1962) indicate that a disturbance of DNA synthesis may be needed for induction. However, such interference by aminopterin can apparently only take place in a certain physiological state of the bacteria, possibly depending on the interelationship between relative rates of DNA - RNA synthesis, an interelationship which changes considerably when there is a shift from one medium to another (Kjelgaard et al. 1958).

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REFERENCES

Adams, M.H. Methods in Medical Research, Year Book Publishers, Chicago, Illinois, 2, 1 (1950).

Ben-Gurion, R. Nature, In Press.

Ben-Gurion, R. and Hertman, I. J.gen.Microbiol. 19, 289 (1958).

Jacob, F. and Wollman, E. Bacteriophages, Interscience Publishers Ltd., London, p. 392 (1959).

Kjelgaard, N.O., Maaløe, O. and Schaecter, M., J.gen.Microbiol. 19, 607 (1958).

Lwoff, A. Bacteriol.Revs. 17, 269 (1953).

Melechen, N.E. and Skaar, P.D. Virology 16, 21 (1962).

Fig. 1 presents graphically experiments of induction with and without thymin, under ordinary conditions of growth and when the cells are transferred from one medium to another.

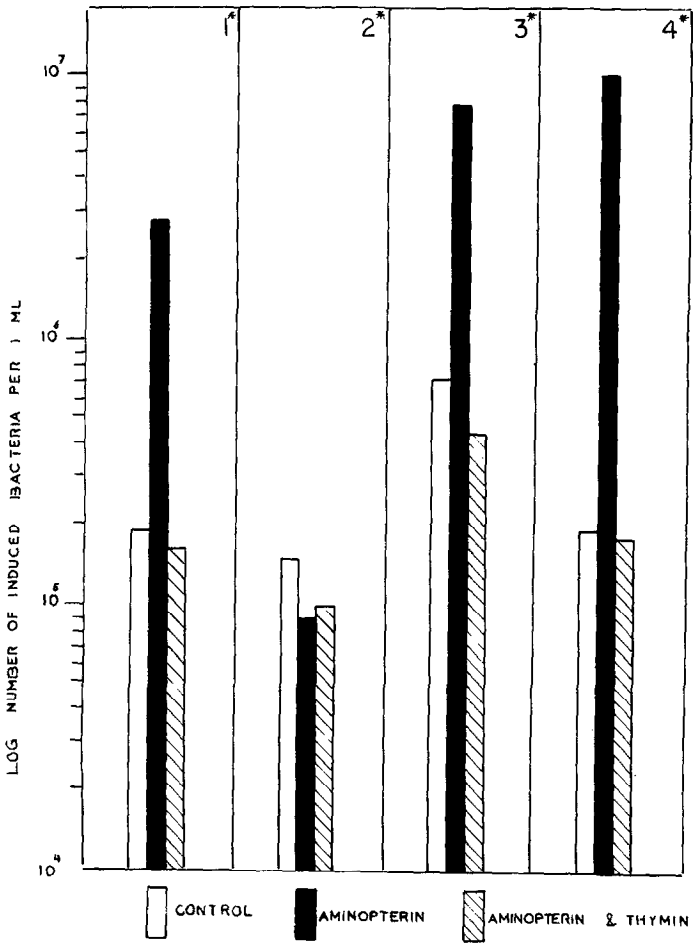

Fig. 1 Effects of thymin on induction by aminopterin

Each column represents the average of three experiments. numbers correspond to suspensions 1, 2, 3 & 4 in Tables 1 & 2.