RECOVERY FROM "THYMINELESS DEATH" IN *ESCHERICHIA COLI* 15T⁻

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When thymine requiring auxotrophs are deprived of thymine but supplied with all their other growth requirements, they lose viability in a characteristic way (Cohen and Barner, 1954, 1955, 1956). This "thymineless death" has been the subject of experimentation and speculation since the time of its first description, but no satisfactory explanation for it has been given. Most of this work has been done with strain 15T⁻ which produces a lethal factor to which it is itself sensitive (Ryan *et al.*, 1955). Since this factor is a protein, it was assumed to be a colicin and called "col_{15}". Later work has shown that "col_{15}" activity is associated with the presence of defective phage structures (Endo and Ayabe, 1965; Sandoval, 1965; Mennigman, 1965). Sicard and Devoret (1962, 1964) and Mennigman (1964) have shown that the induction of this and other prophages is associated with thymine starvation in 15T⁻ and K12T⁻ and have concluded that a part of the loss in viability during thymine starvation is the result of this phage induction. However they have also shown that this cannot be the sole cause of thymineless death, since cells die in the absence of prophage induction.

We wish to report here our finding that, in the absence of prophage induction, "thymineless death" is completely reversible. The observed death of thymine starved cells *follows* their plating onto agar for viable counts and does not occur if the cells are not plated. The "thymineless death" of thymine starved cells therefore appears to be a sensitivity of the cells to being spread on agar.

Strains

E.coli 15T⁻(col_{15}⁺) was obtained from K.W.Fisher, and 15T⁻ "JG151" (col_{15}⁻) from P.Barth. Ishibashi & Hirota (1965) derived JG151 from the survivors of two successive rounds of thymineless death of 15T⁻. This derivative has the same thymine requirement as the parent 15T⁻ but the defective phage "col_{15}" is no longer produced by it. (JG151 may in fact still carry this phage (P.Barth, personal communication), but it is not induced by thymine starvation in this strain.)

Plating was carried out by spreading diluted aliquots onto the surface of plates containing L-broth (per litre, 10gms Difco tryptone, 5gms yeast extract, 10gms NaCl, pH 6.8), 10μgms/ml thymine and 1.5% agar. The samples were spread with a glass rod until all the liquid was absorbed by the agar. Cultures were grown with rotary shaking at 37° in M9 (Nagata,T., 1963) salts with 10μgms/ml thymine and 0.2% glycerol. In one experiment cells were plated onto 1.5% agar supplemented with M9 salts, thymine and 0.2% glucose.

Results

Cells growing logarithmically in M9 plus glycerol plus thymine were washed thoroughly with M9 salts on a membrane filter. They were then re-suspended in M9 plus glycerol, without thymine, and shaking continued at 37°. In the presence of thymine, the generation time of both strains was 60-70 minutes. In the absence of thymine, viability decreased in both strains in approximately the same manner. For the first 20-30 minutes viable counts were constant and then decreased at a first order rate. After 70 minutes, the viable counts of both strains had decreased to about 20-30% of their initial value. (Ishibashi & Hirota, 1965, reported that JG151 lost viability at a much slower rate than the parent 15T⁻, but we did not find this to be the case. Since they gave no details as to growth rates or plating methods, no explanation can be suggested for this.)

In one experiment JG151 was plated onto M9 agar with thymine and glucose. In this case, viability was lost at a much lower rate than in duplicate samples plated from the same culture onto L-agar plus thymine. The number of viable counts on M9 plates was also higher than on rich plates at 0 minutes of thymine starvation. (See figure 1) This difference in apparent death rate on different kinds of plates suggests that the observed loss of viability in thymine starved cells depends on the conditions of plating rather than on death of the cells in the liquid medium.

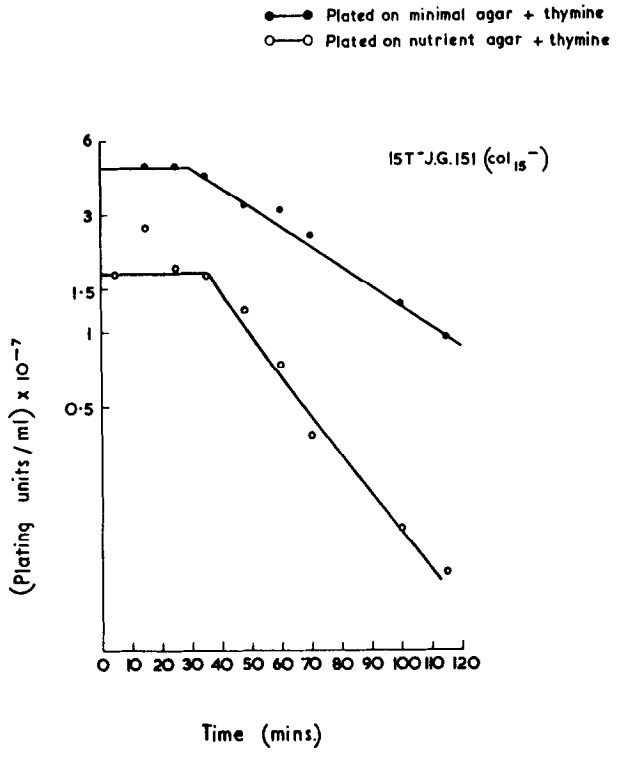

Figure 1 Decrease in numbers of cells capable of giving rise to visible colonies after plating on M9, glucose, thymine agar (upper curve) and L-broth, thymine agar (lower curve) during growth in M9 salts plus glycerol but without added thymine. The strain used was JG151.

The reversibility of this acquired sensitivity to plating can be shown, at least for JG151, by adding thymine to the starving culture after 70 minutes. Figures 2 and 3 show the viable counts obtained on plating $15T^{-}(col_{15}^{+})$ and

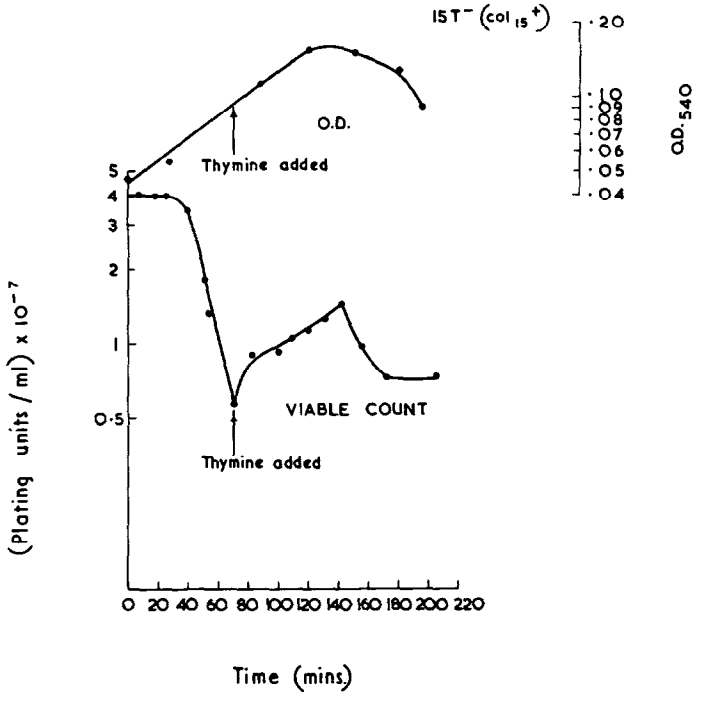

Figure 2 Numbers of cells capable of giving rise to visible colonies after plating on L-broth, thymine agar. $15T^-(col_{15}^+)$ growing in the absence of thymine on M9 salts plus glycerol for the first 70 minutes. 10μgms/ml thymine was added at 70 minutes. The optical density of the culture at 540 millimicrons (1cm path) is shown at the top.

$15T^-JG151(col_{15}^-)$ during thymine starvation and after re-addition of thymine.

In $15T^-(col_{15}^+)$, those cells which can still survive plating after 70 minutes recommence growth after the addition of thymine. However, as can be seen from the second drop in viable counts, many of these survivors are subsequently killed in the liquid culture. Since this second drop in numbers coincides with visible lysis of the non-viable majority of the cells, it is probably the result of killing by released phage (i.e. "col_{15}"). This can occur since strain 15 carrying the defective phage is itself sensitive to killing by it (Ryan et al., 1955). (Figure 2)

In contrast to this, readdition of thymine to JG151 is followed by a rapid and complete recovery of viable counts. 35 minutes after the re-

addition of thymine (at 70 minutes) the viable count is again restored to the initial level present at the time of thymine removal. The number of viable units continues to increase rapidly until it reaches about 50% of the number in an unstarved control culture. Subsequently counts continue to increase at the normal rate for this strain. (Figure 3)

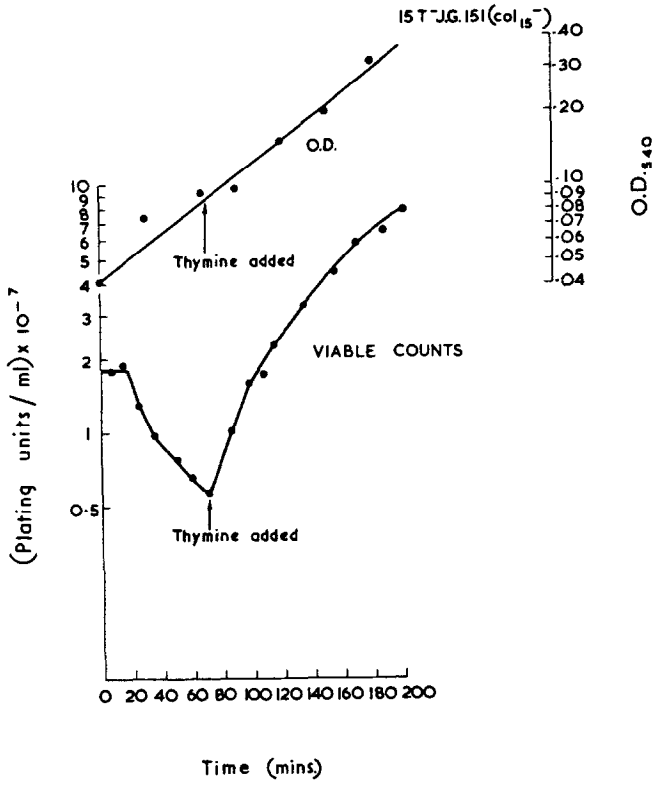

Figure 3 Same as figure 2, except that strain 15T⁻ JG151 (col_{15}^-) was used.

The difference in recovery of the two strains from a period of thymine starvation therefore seems likely to stem from the induction of the defective phage in one but not in the other. The similarity of the curves for loss of plating viability during the period of thymine starvation however shows that "thymineless death" is not itself caused by phage induction. The increasing sensitivity to plating during thymine starvation, which "thymineless death"

can now be seen to be, cannot involve any irreversible changes to DNA or other cell components.

A detailed analysis of the process of recovery from thymine deprivation will be published elsewhere. This will include a study of DNA synthesis and its relation to cell division.

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