# INDUCTION IN ESCHERICHIA COLI 15 OF THE COLICINOGENIC FACTOR BY THYMINE-LESS DEATH.

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The thymine-requiring strain E.coli 15 $T^-$, when deprived of thymine, in a characteristic time-dependent manner loses its ability to form colonies on nutrient agar ("Thymine-less death"; (4)). On the other hand, this same strain is said to be "colicinogenic" (7): on further incubation of an UV-irradiated culture its o.d.[+)] later decreases due to lysis through production of an agent which is assumed to be a colicin. (This agent in several respects behaves like a colicin; but usually production of a colicin is not accompanied by lysis (1).) Both these phenomena - tld and colicin production - now turned out to be correlated, even though after removal of T from the growth medium of a culture of the $T^-$ strain, no such decrease in o.d. can be observed; it rather increases (4).

In this paper we wish to present data showing that T-deprivation of E.coli 15 $T^-$ will eventually induce the culture to produce colicins, and thus bring about lysis, if T is later re-introduced. We therefore conclude that the loss of colony-forming ability of a T-deprived culture is at least partially due to colicin production

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+) o.d. = optical density; v.c. = viable counts; T = Thymine; FUdR = 5-Fluorodeoxyuridine; FUdRP = 5-Fluorodeoxyuridine monophosphate; tld = Thymine-less death.

when the culture is plated on nutrient agar for counting surviving cells.

Experimental The bacterial strains E.coli 15 and its T-requiring mutant E.coli 15 $T^-$ were obtained through the courtesy of Professors F. Ryan and O. Maaløe, respectively. The bacteria were grown in a synthetic medium (4) of pH 7.25, supplemented with both 1.5 % Difco casamino acids and 2 x $10^{-5}$ M T. Experiments were started with a logarithmically growing culture of about 2 x $10^8$ cells/ml. Bacterial assays were performed on nutrient agar plates by the agar layer technique. O.d. readings (1 cm path length) were taken every 10' in the Eppendorf spectrophotometer equipped with a 578 nm filter. To remove the T supplement, the culture was filtered through a collodion filter (filter type no. 3, Membranfilter-Gesellschaft, Göttingen, Germany), washed on the filter with pre-warmed medium and finally re-suspended in the original volume of fresh medium without the T supplement. Where desired, FUdR (a generous gift from Deutsche Hoffmann - La Roche AG., Grenzach, Germany) or T was added from a solution 100 times more concentrated to give 6 x $10^{-5}$ M or 2 x $10^{-5}$ M, respectively. The amount of colicin produced was estimated by the method of Mukai (7). Microscopic observation (on a thermostatted stage) was performed by placing a drop of the culture on a pre-warmed nutrient agar block (1 mm thickness, mounted on a microscopic slide).

Results When the T is removed from a culture of E.coli 15 $T^-$ in T-supplemented medium, after a lag period of about 30' the number of survivors will decrease (4) with a rate constant k of about -o.15 $min^{-1}$. After the v.c. has dropped by about a factor of $10^4$ the curve flattens out (fig. 1, "v.c."). The o.d. first increases at a rate constant characteristic for a non-deprived culture, but becomes

constant when it has approximately doubled its value (fig. 1, O). At no time during this tld (measured up to 8 hrs.) are colicins detectable in the culture.

If now aliquots of such a culture, at various times after onset of T-less conditions, are re-supplemented with T and further incubated, o.d. readings will give a family of curves as presented in fig.1. As one can see, an almost normal increase in o.d. takes place if T is re-introduced before onset of the drop in v.c. (fig.1, A, B, a.C). On the other hand, if T is introduced after this point, the culture will eventually lyse - indicated by a decrease in o.d. - to an extent which is greater, the later T is introduced. (The second increase in o.d. is due to multiplication of those cells which would have formed colonies when plated on nutrient agar.) The latter holds true up to the point where the rate of decrease in v.c. itself decreases. Re-introduction of T from here onwards has only a small effect on o.d. - All these cultures were tested for colicins produced: the more pronounced the lysis was the more colicins could be detected.

In order to find out whether or not this lysis of a liquid culture can be related to what happens when T-deprived cells are plated on nutrient agar, microscopic examination after plating has been undertaken. Generally speaking, the same can be said about the number of cells which will eventually completely lyse, as has been said above about the degree of lysis of a liquid culture and of colicin production. The other cells can roughly be grouped into four classes: (1) they will form colonies, (2) they show no detectable alteration, (3) they will vacuolize, and (4) their entire cytoplasm will form a ball-like structure within the apparently unchanged cell wall. All four classes together amount to at least a few per cent.

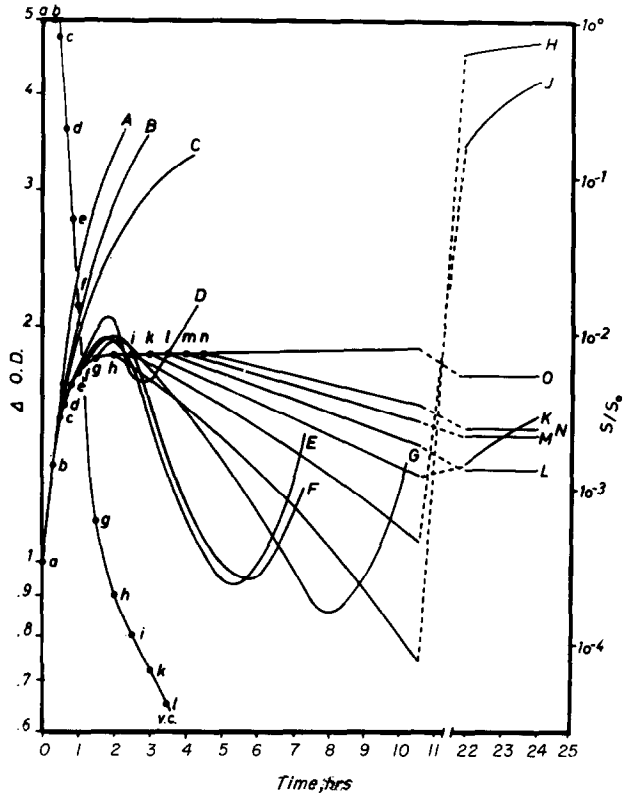


Fig. 1 Effect on optical density of re-introduction of thymine to a thymine-deprived culture of E.coli 15 $T^-$.

At 0 hrs T was removed. Curves "v.c." and "O" indicate changes in viability counts and optical density, respectively. Small letters a - n along curve O indicate when T was re-introduced to an aliquot of this culture. Capital letters A - N mark corresponding o.d. curves. Small letters along curve v.c. indicate fraction of survivors at time when T was re-introduced.

Essentially the same results were obtained when instead of removal of T from a culture of E.coli 15 $T^-$, FUdR was added to a culture of 15 $T^+$. Only the lag period prior to the decline in v.c. was prolonged by a few minutes (probably the time necessary to convert FUdR into FUdRP, the substance which inhibits the thymidylate synthetase (2)).

Discussion The experimental results clearly seem to indicate that by T-deprivation or by chemically blocking the enzyme thymidylate synthetase the colicinogenic factor of E.coli 15 $T^-$ or $T^+$, re-

spectively, can be brought to such a state that it will be able to govern the production of colicins when T is re-introduced to the medium. The above experiments do not show whether or not the induction of the colicinogenic factor is a necessary prerequisite for tld (the presence of T is, however, a necessary prerequisite for the production of colicins (5)). At least as deduced from microscopic observations lysis of cells is not the only reason for loss in colony-forming ability. Thus, it is possible that under conditions of T-deprivation there are two series of events which lead to loss in viability, one being due to the induction of an episome and the other to something which may be named "intrinsic tld". This picture is in agreement with the following observation (5): if tld is examined under strictly controlled pH conditions in the pH-stat (because the rate of death is very sensitive to changes in pH) the v.c. follows a broken curve rather than a smooth one (fig. 1, v.c.). Therefore the picture can be re-phrased: the first decline in colony-forming ability reflects the kinetics of events which are connected with colicin production, and the second one with those of "intrinsic tld". A proof of this picture could be expected from experiments with a strain which is definitely cured of all inducible episomes (5).

Parallel to these studies we continued the investigation of tld by FUdR in Bac.subt.(6). These were stimulated particularly by the recent finding (8) that this strain too contains an inducible episome: after a short-time Mitomycine treatment a defective phage is produced. Studying this strain in experiments similar to the ones described here for E.coli 15, we could show (5) that the production of this phage is also induced when DNA synthesis is temporarily halted by the action of FUdR on the wild type strain or by T-deprivation in a culture of a T-less mutant.

Conclusion We feel tempted to interprete the data as meaning that under T-less conditions first the episomic element (3) "colicinogenic factor" of E.coli 15 $T^-$ (7) in a considerable portion of the culture is changed into an "induced state" and that only later when T is re-introduced to the medium can it express its ability to govern the production of colicins. Thus, under the usual conditions for observing the kinetics of thymine-less death the actual "death" of the non-surviving fraction of the population does not occur until the cells are plated on nutrient agar.

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