THE THYMINELESS DEATH AND DEOXYRIBOSIDELESS DEATH IN LACTOBACILLUS ACIDOPHILUS R-26

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The "thymineless death" has been first observed in Escherichia coli 15 T^- by Cohen and Barner (1954), but the mechanism of this phenomenon has not yet been explained. The hypotheses have been proposed, that the death of cells is due either to the disbalanced growth (Cohen and Barner, 1954), or to the errors in replication of DNA (Maaløe and Hanawalt, 1961) or to the cells inability to repaire the single strand breaks of the chromosome opened during RNA transcription (Pauling and Hanawalt, 1965), or to the induction of a phage (Rolfe, 1967).

Lactobacillus acidophilus R-26 requires another specific precursor of DNA - the deoxyribose, in the form of any of the deoxyribosides (Hoff-Jørgensen, 1952). The absence of deoxyribose results therefore in starvation for all four DNA-precursors. Under these conditions, similarly as in the absence of thymine, the DNA synthesis is absent while the longitudinal growth of the cells

continues up to an 10 - 15 fold increase of O.D.[+)] (Soška and Lark, 1967). This situation offers the possibility to compare the effects of thymine starvation with those of starvation for all specific precursors of DNA. The results have shown that the "deoxyribosideless death" proceeds more slowly than the "thymineless death" and that both phenomena are similarly sensitive to the inhibition of RNA and/or protein synthesis.

MATERIAL AND METHODS

Lactobacillus acidophilus R-26 was obtained from Dr Hoff-Jørgensen in 1957 and has been cultivated in the synthetic medium according to Soška (1966) with the following modifications: The thioglycollic acid was omitted, folic acid was present (0.1 µg/ml) and deoxyinosine (4 µg/ml) was used as the source of deoxyribose. The generation time was 35 - 40 minutes at 39°, the optical density of the culture was measured at 650 nm, the total cell count was determined using the Thoma haemocytometer. The surviving fraction was estimated as colony counts on Petri dishes with a 2% agar -BYG medium containing per liter water: Bactopeptone Difco 30 g, dextrose 15 g, yeast extract Difco 10 g, Potassium acetate 5 g, KH_2PO_4 2 g, Tween 80 1 g. The pH was adjusted to 6.7 - 6.9. Before plating, the culture was serially diluted with a 2.5% KCl solution.

[+)] Abbreviation used: T, thymine; DR, deoxyribose; FU, 5-fluorouracil; Act, actinomycine D; CLP, chloramphenicol; Val, l-valine; R-26, Lactobacillus acidophilus strain R-26; -T, or -DR, medium without thymine or deoxyribosides; O.D., optical density; TLD, thymineless death; DRLD, deoxyribosideless death.

The synthesis of thymine was inhibited by 5-fluorouracil (0.1 µg/ml). This concentration of FU had no influence on the growth of bacteria in the presence of thymine (4 µg/ml) and uracil (10 µg/ml). Increasing concentrations of FU were unable to speed up the death rate. FU was used throughout the experiments whether thymine was omitted or not.

The changes of the medium were performed by the filtration technique of Maaløe and Hanawalt (1961), using membrane filters HUFS (fa VCHZ Synthesia, Semtín, Czechoslovakia) the porosity beeing 0.3 - 0.5 u. The experiments were performed with exponentially growing cultures containing 5 - 10 x 10^6 cells/ml.

The purines and pyrimidines were supplied by the California Biochemical Corporation, deoxyinosine by the Sigma Corp., aminoacids by the Lachema, the vitamines and chloramphenicol by Spofa (both Czechoslovakia), 5-fluorouracil by fa S.A.F.Hoffmann-La Roche and Co., Ltd. Switzerland, and actinomycine D by the Merck Co., Inc.

RESULTS

The death rate occuring during thymine or deoxyriboside starvation is compared in Fig. 1. After a lag of 1 - 2 hours an exponential dying followed, the half-life beeing about 3 times shorter during thymine- than during deoxyriboside-starvation. In both cases the dying was observed to continue through more than six decades. If both thymine and deoxyribosides were omitted at the same time, the bacteria were dying at the slower rate correspond-

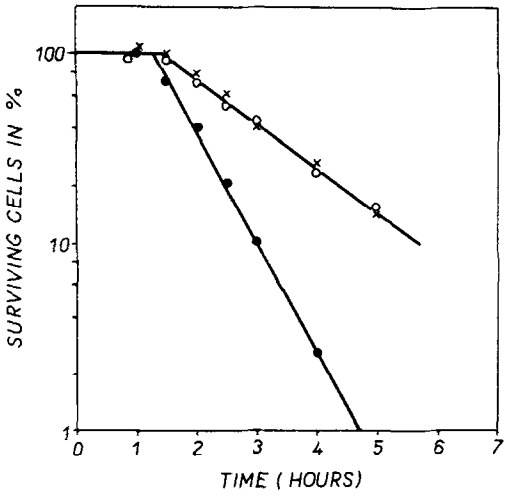

Fig. 1. The thymineless and deoxyribosideless death. An exponentially growing culture of R-26 was transferred into media lacking either thymine (●——●) or deoxyribosides (x——x) or both thymine and deoxyribosides (o——o). At intervals, samples were taken, the total cell count was estimated using the Thoma haemocytometer, the viable count after plating on agar -BYG Petri dishes and the surviving fraction was plotted.

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ing to the situation as found during deoxyriboside starvation. The death of the cells during deoxyriboside starvation will be called as the "deoxyribosideless death" (DRLD). Continuing RNA synthesis (Hanawalt 1963) or protein synthesis (Rolfe 1967) seems to be essential for the TLD in other strains of bacteria. As shown in Tab. I both the TLD and the DRLD were prevented in R-26 if the protein synthesis was inhibited by the absence of one of the essential amino acid (valine) or by the presence of CLP (10 μg/ml). The omission of amino acids results in this strain in the inhibition of protein and RNA synthesis (Soška and Lark, 1966). Both the TLD and the DRLD were

Table I

The effect of inhibitions of protein and RNA synthesis on the TLD and DRLD

Time of incubation	Surviving fraction (in percent) after incubation in media[x)]			
Hours	-T	-T-Val	-T+CLP	-T+Act
0	100	100	100	100
2	25	100	100	96
4	1.7	100	100	90
6	0.11	100	100	82
Hours	-DR	-DR-Val	-DR+CLP	-DR+Act
0	100	100	100	100
2	65	100	100	97
4	23	100	100	90
6	8.3	100	100	88

x) An exponentially growing culture of R-26 was transferred into media as indicated in the table. At intervals samples were taken and the surviving fraction was estimated.

prevented in the presence of Actinomycine D (1.0 μg/ml): this concentration was found earlier to inhibit the RNA synthesis by 90% (Soška and Lark 1966).

DISCUSSION

The results can be summarized as follows:

1) The cells of the strain R-26 die exponentially during thymine starvation as well as during deoxyriboside starvation. In the latter case the death rate is approximately 3 times slower, than the death rate in the absence of thymine only.

2) The thymineless death and the deoxyribosideless death do not differ with respect to their dependence on the

protein synthesis or RNA synthesis.

This similarity could indicate that also the mechanism of both processes are similar. But the slower death rate in the absence of all deoxyribosides than in the absence of thymine only is hard to explain and to reconcile with some of the models of TLD mentioned above. A simple inhibition of DNA synthesis resulting in disbalanced growth or the prevention of repair of the single strand breaks can be used only with difficulties as explanations of the TLD in our case. On the other hand, it appears that the rate of the TLD is influenced, directly or indirectly, by the presence of the other precursors of DNA which may activate or inhibit a process resulting in the death of the cell.

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