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Cell division during inhibition of DNA synthesis in E. coli TAU

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Biofisica. — *Cell division during inhibition of* DNA *synthesis in* E. coli *TAU* (*). Nota (**) di ANNA MARIA SALVATORE, presentata dal Corrisp. M. AGENO.

RIASSUNTO. — Si presenta uno studio del comportamento di cellule di *E. coli* incapaci di sintetizzare timina, durante i primi minuti che seguono al trasferimento dei batteri in un mezzo di coltura mancante di timina.

Si osserva che nel mezzo (— T) una frazione di cellule continua a dividersi per un intervallo di tempo della durata di 10–12 minuti ad una velocità uguale a quella pre shift. Lo stesso comportamento si osserva quando si produce nelle cellule il blocco della sintesi del DNA mediante trattamento con acido nalidixico.

I risultati ottenuti indicano che le cellule capaci di dividersi nel mezzo (— T) sono quelle che al momento del trattamento sono nella fase D del ciclo cellulare e che il periodo D ha, per i batteri del ceppo TAU, una durata molto breve.

When *E. coli* cultures are exposed to ultraviolet light, mitomycin C or nalidixic acid, the DNA synthesis stops immediately, but the cell division goes on for at least 20 minutes after the beginning of treatment [1]. In all the T^- cells undergoing thymineless death an immediate block of the DNA synthesis takes place, when the culture medium is deprived of thymine [2–4]. The experimental data found in the literature agree in establishing that the absence of thymine immediately blocks also cell division [2–9].

The inability of T^- bacteria to divide from the first minutes following their passage to the thymineless medium is a surprising and still unexplained phenomenon. The block of the DNA synthesis, in fact, should not inhibit the division of those cells which at the moment of treatment have already completed a round of chromosome replication.

I therefore decided to investigate this point specifically, using culture conditions such as to make an even small increase in the number of cells easily observable.

To this aim, instead of using a procedure of filtration and centrifugation in the passage of the cells from the medium with to the one without thymine, I reduced by dilution the thymine concentration in the medium to a value which does not alter the phenomenology of the thymineless death [10]. This allowed me to reduce the deadtimes and to carry out the counts immediately after the passage from one medium to the other. Secondly, I started from a culture in exponential growth with the smallest possible generation time τ (23 minutes).

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If D is the time elapsing from the end of the chromosome replication to the subsequent cell division, the fraction of cells having two chromosomes in the exponential culture and which therefore divide under nalidixic acid is given by $2^{D/\tau} - 1$. It then increases with decreasing τ and so chosen conditions are the most favorable to evidence possible cell divisions in the medium (— T).

Titrations carried out under these new conditions showed that cells with two chromosomes actually divide in the medium without thymine, exactly as is the case under nalidixic acid. I could also see why this fact had always passed unnoticed: in the T^- strain I experimented on, (and possibly in other similar strains used by other researchers) the period D is particularly short, about 12 minutes, instead of 20–22 minutes as is the case with the usual strains. This makes it extremely difficult to observe an increase in the number of cells in the thymineless medium, unless one operates under particularly favorable conditions, like the ones I selected.

Then one can also exclude the assumption [11] that thymine molecules are in some way involved in the cell division processes.

BACTERIA AND GROWTH CONDITIONS

The bacteria used are *E. coli* TAU [10].

Cultures were incubated aerobically at 37 °C in a synthetic medium M 9 to which thymine (2 μg/ml), L-arginine (100 μg/ml), uracil (10 μg/ml), L-proline (45 μg/ml), L-methionine (45 μg/ml), L-tryptophan (45 μg/ml), L-leucine (20 μg/ ml) for the medium I, in addition to casaminoacids (0.5 %), adenine (25 μg/ml), cytosine (25 μg/ml) and thiamine (1 μg/ml) for the medium II were added.

The number of viable cells was determined from the number of colonies which appeared after incubation at 37 °C on agar. Soft agar was supplemented with thymine, L-arginine, uracil.

In order to remove thymine molecules from the culture medium, bacteria were filtered (Millipore filters, 0.3 μ, 25 mm) or diluted by a factor 2000 at 37 °C in a thymineless medium.

The behaviour of the bacteria after dilution is the same as that observed when the culture sample is filtered and washed with two volumes of sterile medium [10].

RESULTS

Two cultures of *E. coli* TAU in exponential growth in two different media were deprived of thymine (Fig. 1, 2). The generation times of these bacteria in the media I and II are 42 and 23 minutes respectively.

The cell division in the first minutes of absence of thymine is clearly evidenced by the results in Fig. 2; under the reported conditions a number of cells equal to about 60 % of the initial cells continue to divide exponentially or about 12 minutes.

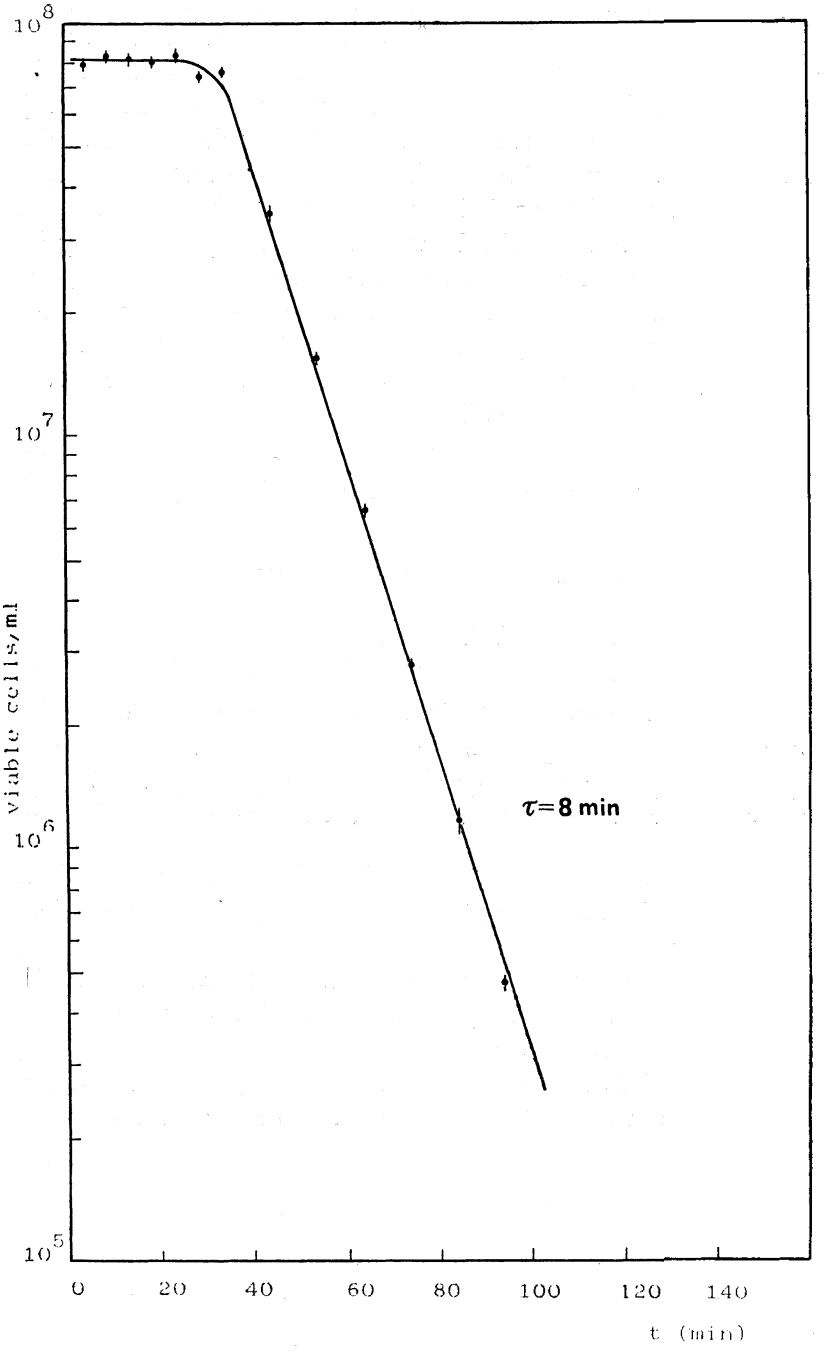

Fig. 1. – Survival of *E. coli* TAU in (—T) medium I. Cells were previously filtered and washed with the same medium deprived of thymine.

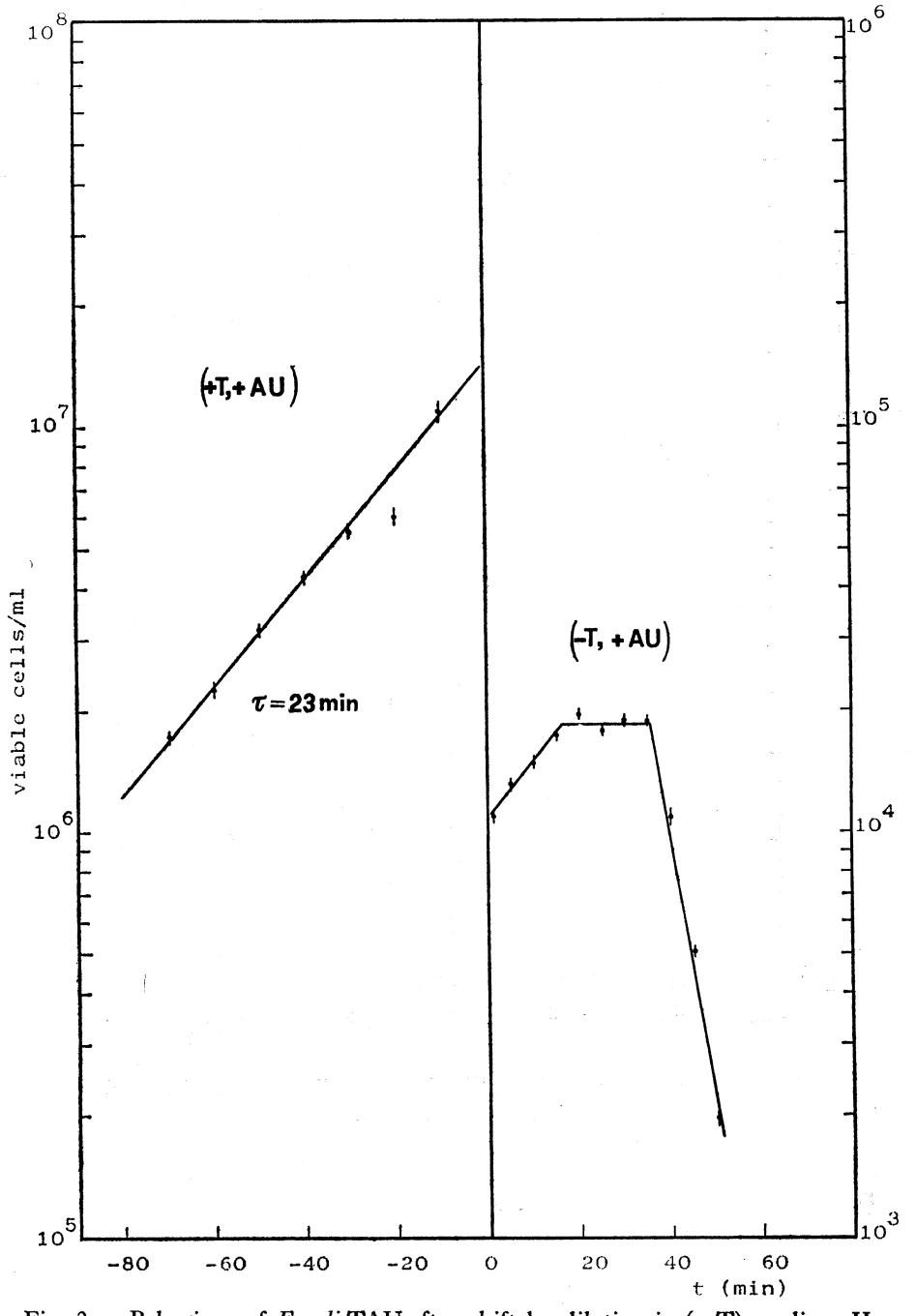

Fig. 2. – Behaviour of *E. coli* TAU after shift by dilution in (—T) medium II.

The behaviour of the cells in the absence of thymine (medium I) is the same as that observed in the first minutes following treatment with nalidixic acid (Fig. 3).

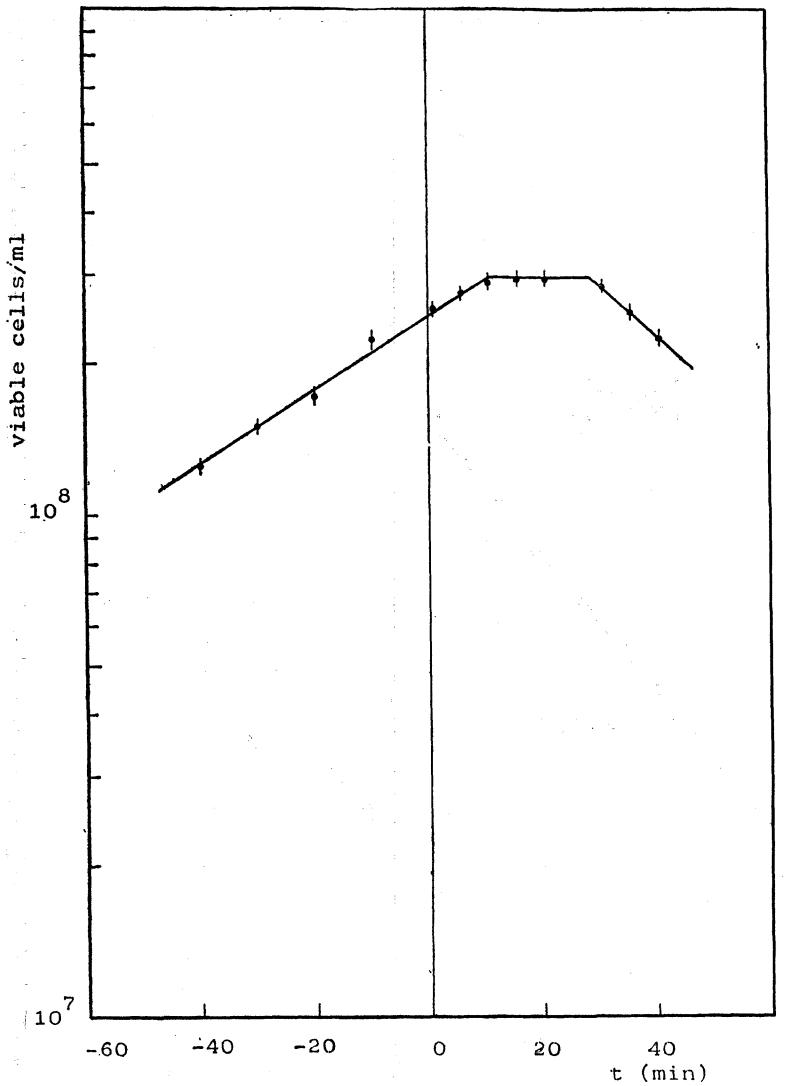

Fig. 3. – Behaviour of *E. coli* TAU after addition of nalidixic acid (7,5 μg/ml) at $t = 0$.

Discussion

The ability of some T^- cells to divide in the absence of thymine indicates that this treatment inhibits the DNA synthesis but does not inhibit, at least initially, other cellular processes, like, for example, the formation of the septum [11].

The number of cells which divide in the absence of thymine is a function of the duplication time of the bacteria. This number turns out to be what can be expected under the assumption that in the absence of thymine all the cells in the D phase of the cell cycle, and only those, can divide [1, 12].

In a population of exponentially growing cells with $\tau = 23$ min, the fraction of cells in the D phase is effectively equal to about 60 % of the total number, in agreement with the experimental result riported in Fig. 2. In the calculations it has been assumed that the duration of the period D in *E. coli* TAU is between 10 and 15 minutes, as shown by the results of Fig. 3.

If, in fact, for the cells in the D phase the cell division is unaffected by the action of the inhibitors of the DNA synthesis [1, 12], D is the period of time during which, starting from the moment at which nalidixic acid is added to the bacterial culture, the cells keep growing exponentially. The duration of this period is also equal to the time interval from the dilution of the cells in (—T) medium to the beginning of the subsequent lag phase (Fig. 2).

Therefore, the results obtained after treatment with nalidixic acid and after removing the thymine are all in agreement and show that in *E. coli* TAU the duration of the period D is constant and very short.

For a slower growth ($\tau \geq 40$ min), the time necessary to change the culture medium makes it impossible to estimate the fraction of cells—much smaller in this case—which can divide.

The result obtained is then in agreement with the conclusion suggested by Helmstetter [1]: the completion of a round of DNA replication is a necessary and sufficient condition for cell division.

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