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Cell lengthening and division in Escherichia coli treated with nalidixic acid

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Biofisica. — *Cell lengthening and division in* Escherichia coli *treated with nalidixic acid* (*). Nota (**) di ARNALDO FLORIO e MARCO SENEPA, presentata dal Corrisp. M. AGENO.

RIASSUNTO. — Lo studio dell'allungamento batterico in colture di *Escherichia coli* trattate con acido nalidixico, ha fornito una conferma indipendente dell'esistenza del processo di divisione cellulare asimmetrica descritto in precedenti lavori.

Con il ceppo batterico usato per i nostri studi, tali divisioni si verificano nel corso stesso del trattamento se questo viene protratto per più di 45 min.

I risultati ottenuti in questo lavoro sono in accordo con il modello interpretativo proposto per spiegare la natura del danno irreversibile che si produce nelle cellule quando il blocco della sintesi del DNA viene protratto per un tempo anche di poco superiore ad un certo valore opportuno.

Different strains of *Escherichia coli* show different sensitivity to nalidixic acid, a powerful bacteriostatic whose mechanism of action is still partially unknown [1]. However the only early effect of relatively low concentration of the drug (5–10 μg/ml, being the threshold concentration for the majority of the strains 2–3 μg/ml) is the rapid and completely reversible block of DNA synthesis [2].

Cultures in exponential growth of *E. coli* treated in such a way, continue to grow for about 20–25 min. from the beginning of the block [3]. During this time interval all the cells which have duplicated their chromosomes before the block (D-phase cells according to Helmstetter [4]) divide. Then the number of viable cells remains constant for about 1 hour and after this time begins to decrease.

In previous papers has been demonstrated that the effect of the drug is not completely reversible if the treatment with NAL is interrupted after a period of time of the same order as the doubling time of the culture [3, 5]. The cells transferred in normal culture medium divide asymmetrically, one daugther cell only being able to form colonies on agar. In order to explain this phenomenon, a model based on the one of Jacob, Brenner and Cuzin [6] for chromosome segregation in procariotes has been proposed. According to these authors the origins of chromosome are permanently attached to the bacterial envelope and segregetion proceeds passively as the bacterium elongates. According to our model, the NAL could determine a tension in duplicated chromosomes, by blocking the replication forks [7]. The mechanical detachment of one of the origin from the envelope, or, more probably the action of an enzyme able to cut the stressed-DNA (topoisomerasis [8]) will ensue.

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The aim of this work was to verify the experimental basis of this model by gathering data of the above mentioned asymmetrical division. We have demonstrated that between 20 and 45 min. of treatment with NAL the cells continue to lengthen but they do not divide. Furthermore we have proved the existence of asymmetrical cell divisions, even in the presence of the drug if the treatment is prolonged for more than 45 min.

MATERIALS AND METHODS

Measurements of bacterial length were made by means of statistical sampling, withdrawn at regular time intervals from a culture treated with 5 μg/ml of NAL. All the cellular activities were blocked and the cells spread on a thin layer, were microscopically measured [9]. For each sample a length distribution has been obtained. The culture growth was monitored both by plating and by microscopically counting the total number of cells.

The strain used is a *E. coli* K12 F′ lac$^+$ proA proB, growing in M9 + glucose 0,5 % + casaminoacids 1,0 % ($\tau = 50$ min.). The total number of cells was monitored with a Petroff-Hausser camera (A. H. Thomas Co. Philadelphia Pa. U S.A.). The samples have been treated with nalidixic acid (Sigma N-8878) 20 μg/ml; chloramphenicol (Serva 16785) 200 μg/ml; penicillin G (Squibb 217 list. 6941) 16 I.U. and fixed with formaldeyde and then spread on a thin layer of polyvinylpyrrolidone (PVP) and agar, containig the same antibiotic mix. The cell length was observed with a phase contrast microscope 2000× (Zeiss ultraphot Universale II), calibrated by use of standard latex microsphere (diameter = 1,001 mm).

RESULTS

In Fig. 1 the growth of the culture as determined by plating (curve a = viable cells) and by microscopic counting (curve b = total cells) is reported.

During the first 40 min. the two growth curves are parallel. Between 40 and 60 min. of treatment the total number of cells increases whereas the viable cell number is constant. After 70 min. the number of viable cells begins slowly to decrease, showing the expected phase of death.

In Fig. 2 the distribution of bacterial length during the first 100 min. of treatment is shown. The distribution at $t = 0'$ is that of an exponentially growing population of *Escherichia coli* [9, 10]. Organisms of unusual length are very rarely observed. The cells in the last phase of division show a narrowing due to the formation of the division septum in the equatorial zone. The cellular diameter appears to be constant. During the first 20 min. sensible variations of length distribution are not observed. At $t = 40'$ the columns of the histogram are shifted toward the higher lengths; simultaneously the class

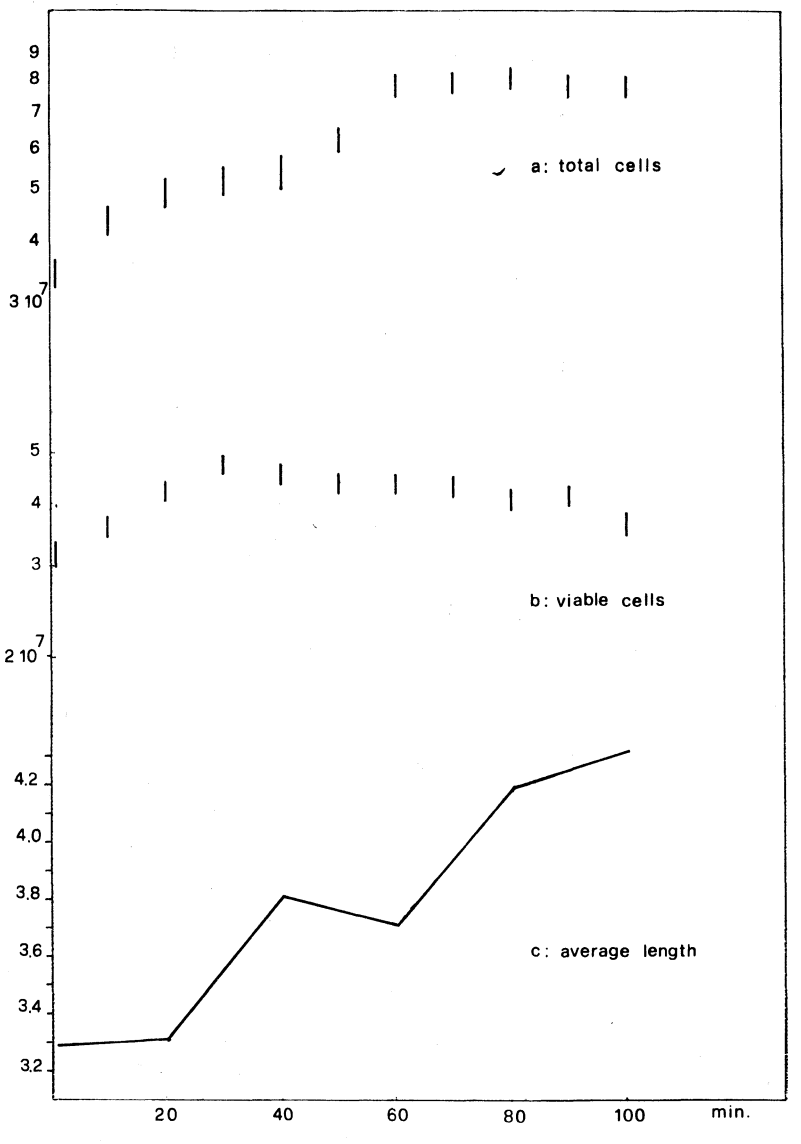

Fig. 1.

of minimum length vanishes. After 60 min. of treatment the short length organisms reappear in the distribution.

The behavior of $\bar{L}$ is reported in Fig. 2 c.

Discussion

The experimental data clearly show that during the first 20 min. of teatment, the organism continues to elongate with the same rate observed during the exponential phase preceding the block. The similarity of the experimental

distribution reported in Fig. 2 a and 2 b is confirmed by the values of average length, practically constant within the experimental errors. As a matter of fact, its value changes from 3,29 mm in the exponential growth to 3,31 mm after 20 min. of treatment with NAL.

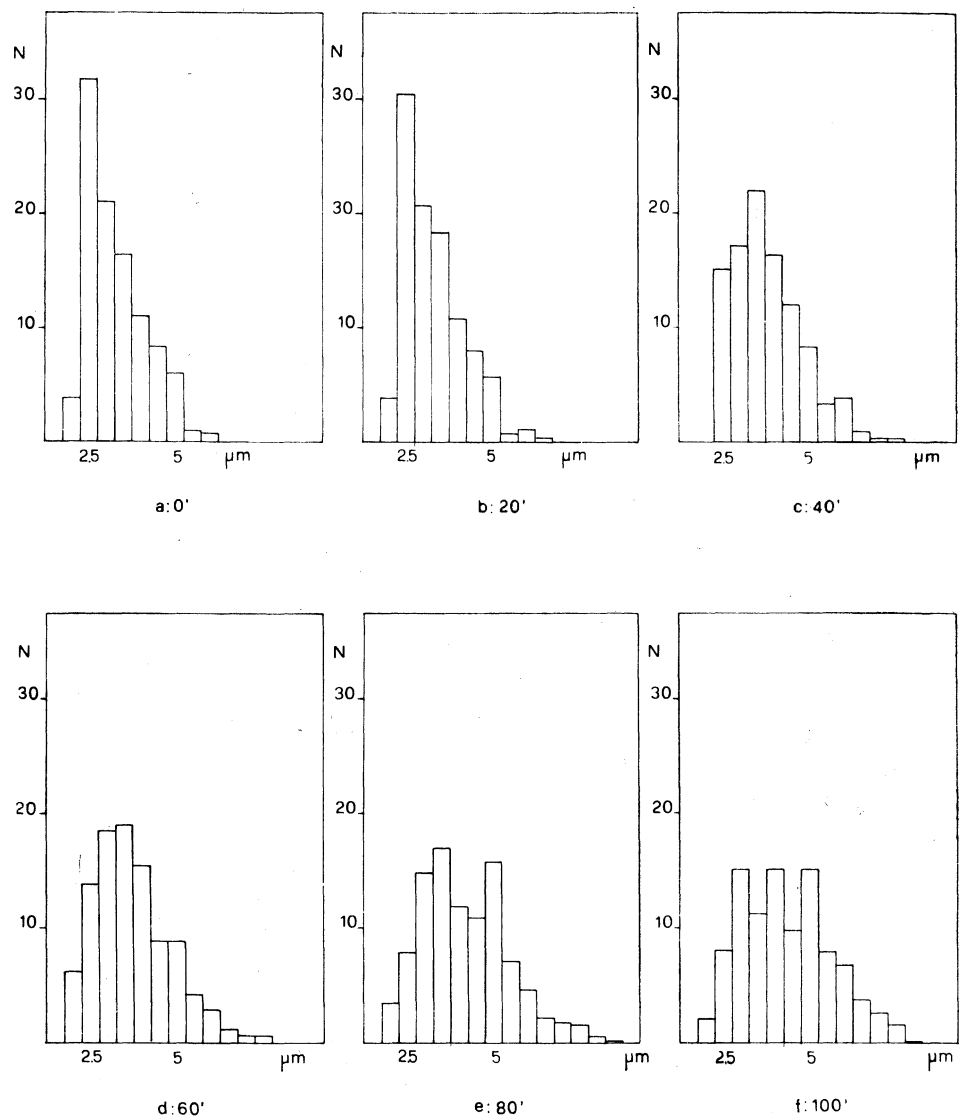

Fig. 2.

In the $t = 40'$ distribution the disappearance of the smallest class of bacteria due to their lengthening and absence of newborn cells is easily observed. On the other hand, after 60 min. of treatment, when cell division starts again, the 2 mm class reappears and afterwards, even if strongly reduced, it still remains in the distribution.

There is good evidence that these newborn cells would be able to elongate only partially, as expected if irreversible damage occurred.

This behavior is clearly due to the asymmetrical divisions as shown in the growth curves in Fig. 1. An analysis of the length distribution confirms our interpretation, excluding the presence of cellular aggregates.

A previous work has described an increase in optical density of the colture during the treatment with NAL, simultaneously with the plateau in the viable cell growth curves [11]. This increase is not linear but seems to level off. This is in accordance with the asimmetrical divisions observed and with the partially inability of the newborn cells to elongate.

The presence of an irreversible damage in this group of cells, lacking of at least part of the chromosome, such as our model predicts, could explain even this last phenomenon.

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