LETHALITY AND INACTIVATION AFTER TREATMENT OF ESCHERICHIA COLI-CELLS WITH NITROUS ACID[+]

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Recently it was demonstrated (Kaudewitz, F., Moebus, K., Kneser, H., 1963) that wildtype cells of E. coli B, incubated in nitrous acid, after plating on nutrient agar give rise to: 1. unchanged wild-type colonies, 2. colonies composed of wild-type and auxotrophic cells (mixed colonies) and 3. colonies consisting of auxotrophic cells only. Experimental conditions provided that samples withdrawn from the incubation mixture were composed of cells harbouring at most one single biologically active nucleus.

The experimental evidence presented is in good agreement with the following hypothesis: Mixed colonies originate from single cells, each of them containing hybrid DNA. This is composed of one subunit changed by deamination probably (Vielmetter, W., Schuster, H., 1960) of a cytosine or adenine and a second subunit with unchanged wild-type composition. In cells producing auxotrophic (type 3) colonies this wild-type strand is mutated lethally by a separate deamination probably of a cytosine or adenine. Under given experimental conditions (Incubation in 0,017 m $NaNO_2$ at P_H 4.3 and 37° C) the average time for one deamination

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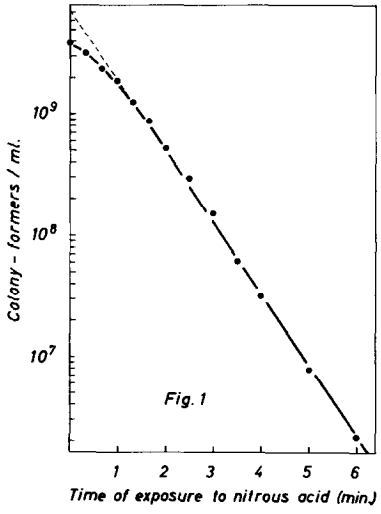
(hit) per subunit leading to lethality is τ_1 = 8,2 min., while that for one hit causing auxotrophy is τ_2 = 4oo min.

This type of analysis does not describe the nature of these DNA "subunits". From data dealing with the kinetics of inactivation of phage by nitrous acid (Vielmetter, W., Wieder, C.M., 1959) and the present knowledge of the reaction of nitrous acid with DNA it is conceivable to consider each of the subunits in question as a single DNA strand within the unit of a double helix.

Cells incubated under the same experimental conditions in nitrous acid loose their ability to form colonies after plating on nutrient agar. This reaction follows an approximate two-target-kinetics (Fig. 1), the final linear part of the semilogarithmic plot of survival vs. time extrapolating to 1,5 - 2 times the original titre. From its slope the average time for one hit extinguishing the ability of a cell to form a colony is estimated as τ = o,7 min. τ is much smaller than τ_1. In addition to that type of hit leading to lethality there must be involved another different type of hit causing "inactivation". Its time constant is τ_3, the combined "killing" following, in the linear part, the kinetics $e^{-t} = e^{-(t/\tau_1 + t/\tau_3)}$. From this equation, with τ_1 = 8,2 min., τ_3, the time constant for inactivation, can be calculated as o,77 min.

The kinetics of mutagenesis (Fig. 2) shows, that even in the linear (one-target) part of the inactivation-curve ($t > 1.5$ min.) the cells contain two subunits which can separately be mutated or hit lethally (with average times per hit τ_1 and τ_2), and jointly inactivated (τ_3). This demonstrates that, at the beginning of the inactivation, there must be present per nucleus more than two such subunits in order to explain the (approximate) two-target-kinetics of inactivation at the beginning of incubation in nitrous acid. Data from work in progress in this laboratory (Kaudewitz, F., Moebus, K., Kneser, H., in preparation) are in agreement with the hypothesis that in resting cells there are not present four complete single or two double-stranded units per nucleus, but

one DNA double strand instead, which in separate cells is in different stages of duplication.



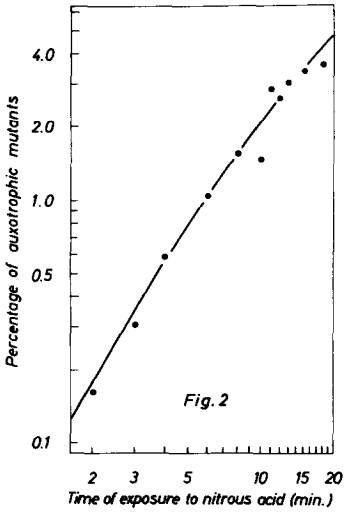


Fig. 1 Relationship between the time of exposure to nitrous acid (o,o17 m $NaNO_2$, pH 4.3, 37°C) of resting cells of E.coli B and their loss of the ability to form colonies after plating on nutrient agar.

Fig. 2 Relationship between the time of exposure to nitrous acid (o,o17 m, pH 4.3, 37°C) and the percentage of cells induced to form auxotrophic (non-mixed) colonies. A cell only then gives rise to such a colony (Kaudewitz, F., Moebus, K., Kneser, H., 1963) when it harbours a DNA double helix, one strand of which was hit lethally by a single deamination, the other one obtained a separate hit (deamination) leading to auxotrophy. This is reflected by the kinetics of induction to auxotrophy: When both axes of the plot have the same logarithmic scale the slope of the curve for t = 1.5 min. is about 60°. It only decreases very slowly with growing t, thus indicating for the time interval under experimental investigation a close approximation to two-target-kinetics.

Hence, briefly summarized, two types of events destroy the ability of nitrous acid incubated cells of E.coli to form colonies: 1. The deamination of most probably one cytosine or adenine in one of two DNA sister strands leads to "lethality" of only that cell or the clone derived from it which harbours this single strand and its newly synthesized sisterstrand. 2. The deamination of most probably (Vielmetter, W., Schuster, H., 1960) one guanine in one of two DNA sister strands leads to "inactivation" of both strands.

Type-1 events produce cells which genetically are lethals. They may be defined as auxotrophs with growth factor requirements which are not satisfied by nutrient agar. Their enzymatic outfit originating from the time before treatment with nitrous acid enables them to maintain biosynthesis for a limited period of time. It ceases during the following generations by the processes of diluting-out during cell-divisions and metabolic decay of the type of enzyme in question. Such a cell produces a microclone at most. Because of their wild-type enzymatic outfit, cells lethally hit by nitrous acid also should be able to proceed synthesising DNA for a limited number of generations. Experiments were done to test if this is also true for cells "inactivated" by a type-2 event.

Since inactivation is about 1o times more frequent than lethality, cell suspensions were used which had been treated with nitrous acid before, and no attempts were made to separate inactivated cells from lethals. Experiments were done by use of a thymineless mutant of E.coli B 3o7 c isolated in this laboratory. The experimental procedure excluded thymineless death. Cell suspensions, which had been incubated with nitrous acid, were washed in the cold and inoculated into minimal medium supplemented with 15γ/ml Thymine + C^{14}-labeled Thymine. At time intervals samples were withdrawn, treated with 1o % icecold trichloracetic acid, and collected on a membrane filter. The activity was measured by means of a windowless gas-flow counter. About 5o % of the cells used were uninuclear.

Fig. 3 curve C presents data obtained with cells of a suspension inactivated to a survival rate of o,1 %. At this low percentage of survival, DNA synthesis appears to be diminished to a comparably low order of magnitude, i.e. it is hardly detectable.

A more detailed analysis of this finding is possible by use of cell suspensions with much higher survival. Fig. 3 curve B is plotted from data obtained with the same technique as above, however, with a survival of about 36 %. In such cell suspensions at the time of inoculation

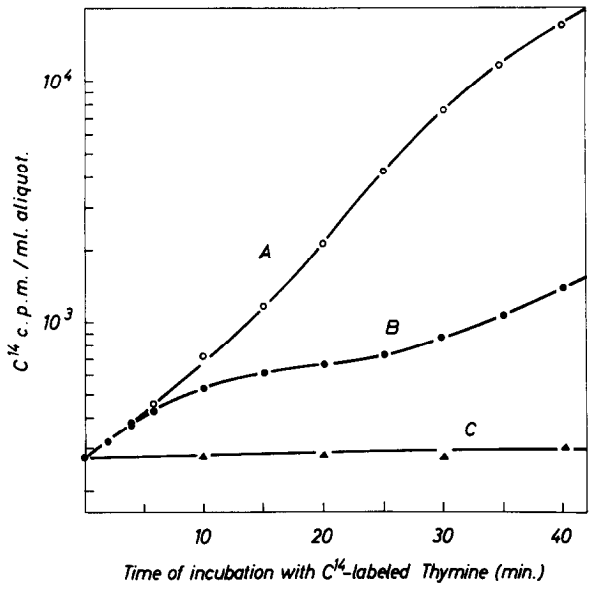


Fig. 3 Effect of inactivation of resting E.coli cells by nitrous acid on their ability to incorporate C^{14}-labeled Thymine as a measure of DNA synthesis. A. Control, not treated with nitrous acid. B. Cells inactivated to a survival of 36 %. C. Cells inactivated to a survival of o.1 %.

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into broth the rate of DNA synthesis is equal in the untreated (curve A) and treated (curve B) sample. With progressing time the latter shows a decreasing rate of synthesis leading to a minimum at about 2o min., which is the time, when in the control the DNA content is doubled. (This was shown by data from direct estimation of DNA not given here.)

With a survival of 36 % most of the cells obtained only one hit leading to inactivation. Hence these findings may be interpreted in the following way: Duplication of DNA starts in a zipperlike fashion from one end of the double stranded DNA molecule harboured by one nucleus. It is irreversibly blocked when in one of both sister-strands a site is reached, in which an inactivating hit has occured before, i.e. a site where most probably one guanine was deaminated to xanthine. From 0 to 20 min. the slope of curve B mainly reflects the random location of such sites in different DNA molecules. Beyond 2o min. DNA synthesis no longer takes place in inactivated cells. It continues however for a limited

period of time in cells lethally hit and it goes on unchanged in cells with undamaged DNA. A quantitative analysis of this hypothesis is under way.

ACKNOWLEDGEMENTS

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