The Effect of Nitric Oxide on the Growth of *Escherichia coli* M.

During investigations into the role of nitric oxide (NO) as a modifier of radiation sensitivity[1,2], it was noticed that the size of colonies which arose from organisms previously treated with NO was smaller than that of control colonies, although the viable count of the bacterial suspensions was not reduced. It was therefore decided to examine whether NO influenced the characteristics of growth, i.e. the effect of prior treatment with NO on the growth-curve of organisms subsequently grown in liquid medium.

Experimental. The organism used was *Escherichia coli* M. Suspensions of stationary phase bacteria in phosphate buffer, pH 6.8, were prepared as described earlier[2]. Aliquots containing approximately 10^7 cells/ml were subjected for 60 min to different concentrations of NO in the apparatus previously used in radiation experiments[1]. After removal of the NO, 2 ml of the suspension were added to 18 ml of nutrient broth (Oxoid), previously brought to 37°C, and a sample immediately removed to give the viable count at zero time. Further samples were removed at intervals up to 4 h, suitably diluted and plated onto triplicate plates of nutrient agar (Oxoid) so that multiplication could be followed. One suspension of bacteria was treated with N_2 in the same apparatus to eliminate any effects due to the gassing procedure.

Results. Figure 1 shows typical growth curves after treatment with various concentrations of NO. As noted earlier the initial viable count was unaffected by NO but the lag phase of treated organisms was longer than that of controls. During logarithmic growth, however, the generation time of organisms treated with NO was the same as that of control cells.

The extension of lag phase could therefore be measured by the displacement of the line of logarithmic growth. Figure 2 shows the relationship between the concentration of NO and this displacement. The extension of the lag phase increased with increasing concentration of NO up to a maximum of about 65% NO.

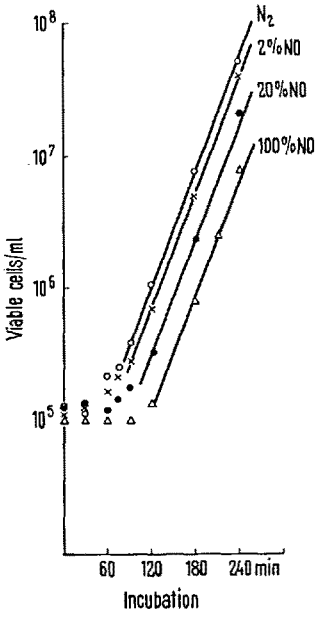

Fig. 1. Growth of *E. coli* after treatment with NO for 60 min.

Discussion. The shape of the curve relating the extension of lag phase to concentration of NO is reminiscent of that showing the effect of pretreatment with NO on subsequent radiation sensitivity with viability as the criterion[2]. In the latter case a plateau was also reached at about 65% NO.

The effect of radiation upon bacterial growth has been studied by ALPER[3,4], who found that the lag phase was extended but that the final generation time was unaffected. JACKSON and WOODBINE[5] showed that sublethal heat treatment of *Staph. aureus* caused a delayed lag phase during subsequent growth, the final rate of multiplication being the same as that of the control culture.

These findings suggest that treatment with NO, radiation, or heat results in damage to the cell which can be repaired or by-passed during subsequent metabolism[6].

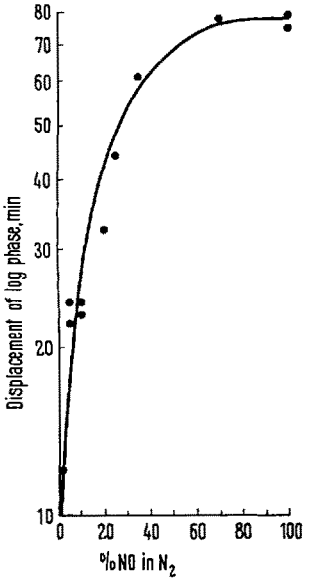

Fig. 2. Extension of lag phase as a function of concentration of NO.

Résumé. Escherichia coli traité au NO présente le même nombre de cellules vivantes et la même courbe exponentielle de croissance, mais la phase latente, qui dépend de la concentration du NO, est prolongée.

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[6] Acknowledgments: The author wishes to thank Mrs. J. HOPKINS for her valuable technical assistance.
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