Induction of Mutations by Nitrous Acid on Denatured
Haemophilus influenzae Deoxyribonucleic Acid Assayed
Directly by Single-stranded Transformation

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The experimental procedure involved the use of DNA carrying a novobiocin resistance marker (C25) which made it possible to follow the inactivation of the treated DNA, as well as the appearance of induced mutations. The DNA was heat-denatured, quickly cooled, treated with nitrous acid, and assayed for the loss of the C25 marker and for the appearance of streptomycin and kanamycin markers in the denatured transformation assay. In addition, portions of the treated DNA were renatured and assayed in the native transformation assay. (See figure legend for details of the experiment). Figure 1A shows the loss of the C25 marker activity as a function of HNO2 treatment as assayed before and after renaturation. These assays were done at saturating concentrations of DNA. The same results were obtained with concentrations in the linear response region. The inactivation of the C25 marker assayed after renaturation was slower than treated DNA assayed directly in the single-stranded system. One possible interpretation of these results is that the inactivated marker which had been renatured undergoes repair inside the cells, whereas single-stranded DNA is not repaired. The dark repair of ultraviolet damage of cells and transforming activity has been described for H. influenzae (R. F. Day, Ph.D. Thesis, Pennsylvania State University, University Park, 1967).

The induction of mutations to streptomycin and kanamycin resistance is shown in Fig. 1B. These results demonstrate that mutations on denatured DNA may be scored directly without renaturation in the single-stranded assay system, as well as after renaturation in the native assay system. The kinetics of mutation induction appeared similar for both assay systems, although the absolute numbers of mutants to both kanamycin and streptomycin resistance was greater for the renatured DNA (Fig. 1B). This difference may be attributed to the one hand to the higher transforming efficiency of native DNA, normally two- to fivefold better than denatured DNA (E. H. Postel and S. H. Goodgal, J. Mol. Biol. 27:247, 1967), and on the other hand to the higher specific transforming activity of the renatured DNA by virtue of its less extensive inactivation (Fig. 1A).

The fact that nitrous acid produced mutations only on denatured DNA in H. influenzae suggested that its action was a direct attack on the exposed bases of the denatured DNA (E. E. Horn and

However, the simplest interpretation suggests that a direct incorporation of DNA with altered bases is responsible for mutations. This conclusion is supported by evidence which demonstrates that the expression and replication of nitrous acid-induced transformants does not differ from that of normal transformants (S. H. Goodgal and E. H. Postel, Science 148:1095, 1965).

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