

Werner Text

The experiments here described are to determine the effect of generation time on the steady state enzyme level of a "perfect cryptic" mutant of *E. coli* at a fixed TMG concentration. The "perfect cryptic" describes an organism in which no galactoside permease can be induced with any~~y~~ galactoside, be it α or β . The organism used was a strain of ML₃ obtained from Dr. M. Cohn. The organism was grown in the Chemostat, limited by 20 milligrams per liter of ammonium chloride. The carbon source was succinic acid; the pH 6.75. Since a perfect cryptic strain has no demonstrable carbon dioxide effect, the gas phase used was atmospheric air. In two experiments the following results were obtained:

In experiment A, the inducer concentration was 2×10^{-4} M TMG, growing at a generation time of 3 hours. The organism reached and held a steady state enzyme activity of 4% of the ceiling. Changing τ to six hours did not alter this value. A further change in the generation time to nine hours did not raise the enzyme level above 4.75%. ^{The} ceiling was obtained at 2×10^{-3} M TMG to the growth tube and tank of the Chemostat when the τ determinations were over.

In experiment B, the concentration of 7×10^{-5} IPG (isopropothiogalactoside) brought the organisms to a level of 5% of the ceiling; varying the generation time from three hours to nine hours gave enzyme levels 5 and 6.1% of the ceiling respectively. When the generation time was altered from nine hours back to three hours, there was again no significant change in the enzyme level.

All enzyme level observations were made for at least four generations at any given generation time.

Strain 2241-X, a perfect cryptic mutant of *E. coli* K₁₂, when grown at 2×10^{-5} TMG reached a level of 1% of the ceiling; the

generation time was three hours, and not more than 1.25% of the ceiling at a generation time of ten hours.

The conclusion is that the rate of β -galactosidase synthesis in a perfect cryptic mutant, at a fixed inducer concentration, is the same per generation over at least a threefold range of generation time.

Experiments were done using strain B and strain ML₃₀ of E coli, growing in the Chemostat with nitrogen limitation, using succinate as the carbon source and 4% CO₂ air-gas phase. To determine the effect of generation time on K, the inducer was thiomethyl-β d-galactoside. K was measured in identical chemostates using 7×10^{-6} M TMG in one pair and 10×10^{-6} in the other pair. One chemostat in each pair was growing at generation time of three hours and one at nine hours. Pair A at 7×10^{-6} TMG gave a K of .005 per generation in both machines.

To confirm the constant nature of K per generation at a fixed inducer concentration, transfer experiments were done in which cells growing at 10×10^{-5} TMG in two chemostats, one at three-hours generation time and one at ten-hours, were transferred at various times to maintenance inducer concentration in test-tubes. After fifty-fold regrowths, the enzyme level of the test tube culture gives us a measure of the number of induced cells at the time the culture was inoculated. The rise in activity of the culture tubes is identical with the one plotted against generations. A third demonstration of the constancy of K over a wide range of generation time is obtained by suddenly altering the generation time of a chemostat growing at three hours to nine hours at low inducer concentrations (7 to 10×10^{-6} TMG). The rise in enzyme and rate of synthesis of enzyme gave an unbroken straight line when plotted against generation time but obviously not when plotted against clock hours. It is concluded from these experiments that the fraction of cells induced per generation at a given inducer concentration is constant over at least a three-fold range of generation times.