SOME OBSERVATIONS ON THE MODE OF ACTION OF COLICIN F.

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It would appear that the lethal action of colicins on susceptible bacterial cells may be separated into two major steps:- namely the initial combination of the colicin with specific receptor sites located on the cell wall of the sensitive organism (Bordet and Beumer, 1951; Fredericq, 1952), which is then followed by some sequence of events which lead to the death of the cell. Using the system colicin F and *E. coli* 58161 we have been able to separate these two steps experimentally, with respect to time.

E. coli 58161, in the logarithmic phase of growth in nutrient broth, was treated with colicin F (2 A.U./ml., Reeves, 1963), and the system maintained at 37° C. At suitable intervals of time the number of cells able to produce colonies on an agar plate was ascertained by the dilution of samples into sufficient nutrient broth to prevent further adsorption of colicin. Similar dilutions were made into a solution of trypsin (5 mg/ml.) and 2:4 dinitrophenol (2 x 10^{-3}M, to prevent growth in the trypsin) at pH 8.0 maintained at 40° C. In the case of the broth dilutions the cells were serially diluted and plated immediately, but the cells were left in contact with the trypsin solution for 20 minutes

before serial dilution and plating. Colony counts (Fig. 1) showed that the inactivation of adsorbed colicin F by trypsin, within the first few minutes of adsorption, can reverse the lethal effect of the colicin. This study was carried further by treating the sensitive cells with 2:4 dinitrophenol (2 x 10^{-3}M) for 30 minutes prior to the addition of colicin and determining the number of cells able to form colonies when samples were diluted into broth and into trypsin solution as described previously. Pretreatment of the cells with 2:4 dinitrophenol permits the reversal of colicin action by trypsin

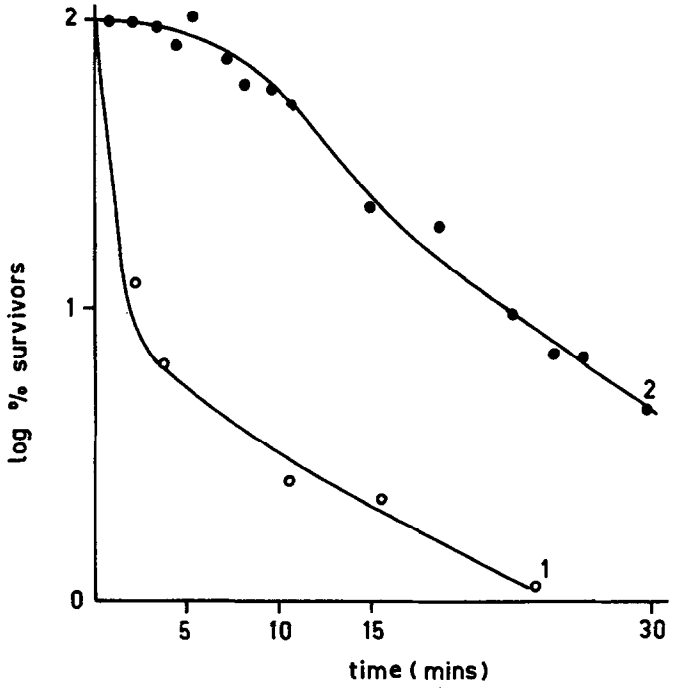

Figure 1. The reversal of colicin activity by trypsin. Log phase E. coli 58161, in nutrient broth at 37°C., treated with colicin F (2 A.U./ml.) at time 0.

Curve 1 - normal viable count.

Curve 2 - viable count performed after dilution into trypsin (5 mg/ml.) at pH 8.0 and 40° C.

for a period of at least two hours (Fig. 2). Such a result is to be expected if the events which take place subsequent to the adsorption of colicin involve energy requiring processes.

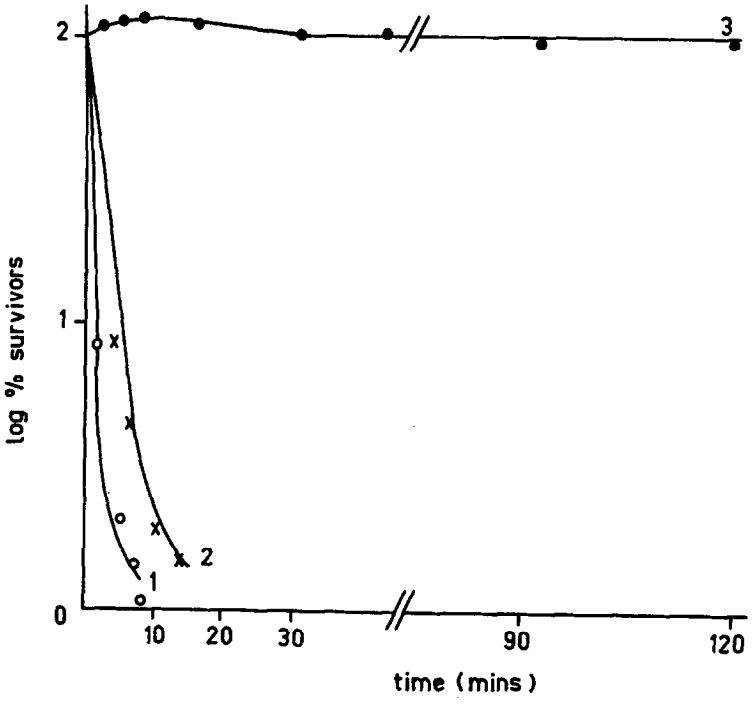

Figure 2. The complete rescue of colicin treated, non-metabolising cells, by trypsin. E. coli 58161 pretreated with 2:4 dinitrophenol (2×10^{-3}M) for 30 minutes prior to the addition of colicin F (2 A.U./ml.) at time 0.

Curve 1 - normal viable count.

Curve 2 - log phase E. coli 58161, in nutrient broth (without DNP) at 37°C., treated with colicin F (2 A.U./ml.) at time 0, normal viable count.

Curve 3 - viable count performed after dilution into trypsin (5 mg/ml.) at pH 8.0 and 40° C.

The effect of colicin F on the synthesis of beta-galactosidase in E. coli K 12 was also investigated. The cells were grown in minimal medium supplemented with casamino acids, sodium lactate and methionine. The synthesis of beta-galactosidase (Fig. 3), could not be induced with methyl beta-D-thio-

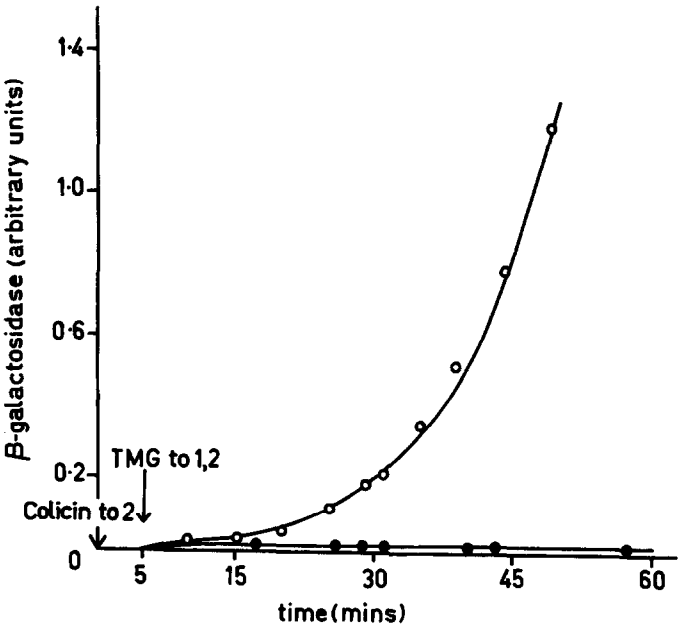

Figure 3. Inhibition of the synthesis of beta-galactosidase in a log phase culture of E. coli 58161 by colicin F (3 A.U./ml.).

galactoside (TMG) when colicin (3 A.U./ml.) had been added to the growing cells 5 minutes previously. However, when the colicin was added to previously induced cells the synthesis of beta-galactosidase did not cease immediately but continued at the same rate as in the control for several minutes (Fig. 4), and did not cease completely until some 15 to 20 minutes after the colicin had been added.

Hence the mode of action of colicin F on E. coli K12 appears to differ in certain respects from that of colicin K on E. coli B (Nomura and Nakamura, 1962), and of colicin ML on E. coli Bordet (Jacob et al. 1952), as in these systems protein synthesis was shown to cease immediately on the addition of the colicin to a growing culture. Nomura and Nakamura (1962) demonstrated the ability of trypsin to reverse, for at least 30 minutes after adsorption, the action of colicin K on macro-

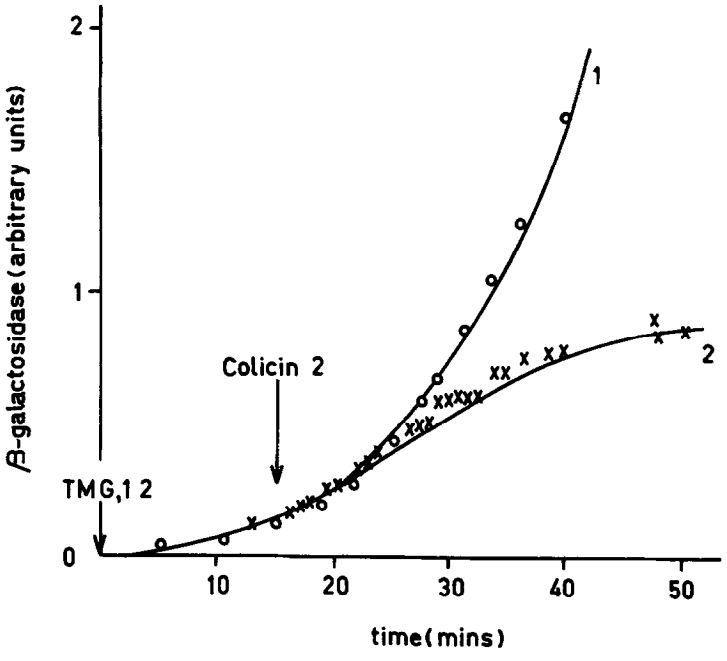

Figure 4. Effect of colicin F (3 A.U./ml.) on the synthesis of beta-galactosidase previously induced in a log phase culture of E. coli 58161.

molecular syntheses. It is possible that the immediate and total inhibition of all macromolecular syntheses by colicin K prevents any gross damage to the cell and permits the rescue by trypsin over relatively long periods of time.

These experiments, with the system colicin F and E. coli 58161, demonstrate that the colicin does not immediately inhibit protein synthesis, that the lethal action of the colicin on growing cells can be reversed by trypsin for several minutes after its adsorption and that the colicin does not kill cells, in which all metabolic activity has been inhibited, for at least two hours after its adsorption.

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