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THE EFFECT OF ISOPROPYLTHIOGALACTOSIDE,  
ON THE INDUCTION OF THE GALACTOSE OPERON  
BY D-FUCOSE IN A LACTOSE DELETION MUTANT  
OF ESCHERICHIA COLI

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D. C. H. McBrien and V. Moses  
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Abstract

The Effect of Isopropylthiogalactoside on the Induction of  
the Galactose Operon by D-Fucose in a Lactose Deletion  
Mutant of Escherichia Coli.

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The gratuitous induction of the galactose operon in  
Escherichia coli by  $5 \times 10^{-3}$  M D-fucose is shown to be in-  
hibited by 43% in the presence of an equimolar concentration  
of isopropyl- $\beta$ -D-thiogalactoside in a mutant of the bacterium  
lacking the lactose operon. This result is compared with  
results obtained by other workers using strains of the bac-  
terium with complete genetic apparatus and possible mechanisms  
of the inhibition are discussed.

The Effect of Isopropylthiogalactoside on the Induction of the Galactose Operon by D-Fucose in a Lactose Deletion Mutant of Escherichia Coli.

by D. C. H. McBrien and V. Moses

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Buttin (1963) has demonstrated that D-fucose (6-desoxy-D-galactose) is an effective gratuitous inducer of the galactose operon in Escherichia coli. However, in the presence of an equimolar concentration of isopropyl- $\beta$ -D-thiogalactoside (IPTG) the induction of galactokinase in mutant 3300 of the bacterium by  $4 \times 10^{-3}$  M fucose was inhibited by 70%. Williams & Paigen (1965) have shown that this repression can be overcome by increasing the concentration of the inducer, induction and repression behaving as competitive phenomena. Moreover, these authors have shown a similar effect of fucose upon the induction of the lactose operon by IPTG. In view of the possibility of an interaction during the simultaneous induction of the two operons, the present authors considered that it would be interesting to investigate the effect of IPTG on the induction of the galactose operon by fucose in a strain of E. coli deleted for the lactose operon; in such a strain an interaction could not occur.

E. coli 2000 X74, a strain carrying a complete deletion of the lactose region (Nagao, Rouviere & Gros, 1965), was grown on glycerol-minimal medium supplemented with arginine, histidine and thiamine. Four parallel cultures were taken whilst the cells were growing in exponential phase at 37°; IPTG and fucose were added as indicated in Table I. Samples were withdrawn from the cultures onto chloramphenicol (100  $\mu$ g/ml) at regular intervals while the cells continued to grow logarithmically as determined

by turbidity measurements at 650 m $\mu$ . The samples were subsequently assayed for galactokinase activity using the method of Buttin (1963), except that the reaction products were separated by one-dimensional paper chromatography using butan-1-ol:propionic acid:water (46.8:32.5:30.7 v/v/v) as solvent. The observed incorporation of [G-<sup>14</sup>C] galactose into galactose-1-phosphate is shown in Table I. From the table it will be seen that the rates of synthesis of galactokinase in cells treated with fucose and with fucose and  $5 \times 10^{-4}$  M IPTG were almost identical, but that with cells treated with fucose and  $5 \times 10^{-3}$  M IPTG the rate of synthesis of the enzyme in excess of that in the uninduced control was consistently inhibited by approximately 43%.

The interactions of IPTG and D-fucose in the induction of the galactose and lactose operons, observed by Buttin (1963) and by Williams & Paigen (1965), can be explained in at least three ways. First, they may be caused by the competition of the two protein synthesizing systems, operating simultaneously, for a limited supply of precursors or cellular apparatus. Second, the induction of one operon, or a product thereof, may act in an unspecified way as an inhibitor of the induction of the other. Third, each inducer may act as a competitive inhibitor in the reaction of the other with its acceptor site in the cell (presumably the repressor). Neither the first nor the second explanation can account for the inhibition by IPTG of the inductive effect of fucose on the galactose operon in a lactose-deleted strain of E. coli, although the possibility of the operation of such processes in cells with a complete genetic apparatus cannot be excluded. Buttin (1963) noted a 70% inhibition in the formation of galactokinase in a lac<sup>+</sup> strain under similar experimental conditions to those which produced a 43% inhibition in 2000 X74. However, these strains

were not derived from the same parents and the disparity in the extent of inhibition may have been determined by parental differences. The third explanation satisfactorily accounts for the results of the present experiment and is in accord with the view of Buttin (1963) that the intervention of IPTG in the regulation of the biosynthesis of  $\beta$ -galactosidase and galactokinase takes place at two distinct cellular sites.

Our thanks are due to Dr. S. Cooper for a stimulating discussion which led to the performance of this experiment. D. McB. acknowledges the receipt of a N.A.T.O. fellowship from the British government. The work reported in this paper was sponsored by the U.S. Atomic Energy Commission.

#### References

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Table I. The assay of galactokinase activity

Each 150  $\mu$ l sample of bacterial suspension was incubated at 37° for 10 min. with 20  $\mu$ l toluene in the presence of magnesium chloride  $10^{-3}$  M, EDTA  $10^{-3}$  M and  $\beta$ -mercaptoethanol  $5 \times 10^{-2}$  M. To each sample was added 100  $\mu$ l of a solution containing 0.25 moles [ $G-^{14}C$ ] galactose (2  $\mu$ c/ $\mu$ mole), 0.4  $\mu$ moles ATP, 0.33  $\mu$ moles magnesium chloride, 0.8  $\mu$ moles sodium fluoride and 8  $\mu$ moles glycylglycine/sodium hydroxide buffer (pH 7.5). The mixture was incubated for 15 min. at 37°, then 50  $\mu$ l aliquots were withdrawn into 400  $\mu$ l 95% ethanol and spotted onto Whatman No. 4 chromatography paper. After development for 20 hr. in butan-1-ol:propionic acid:water (46.8:32.5:30.7 v/v/v) spots were located by radioautography, excised and counted on the paper using two closely opposed end-window Geiger-Muller tubes with a combined efficiency of approximately 15%.

Formation of galactose-1-phosphate (counts/min. per ml suspension)  
corrected for background and co-incidence

concentration of IPTG	-	-	$5 \times 10^{-4}$ M	$5 \times 10^{-3}$ M
concentration of D-fucose	-	$5 \times 10^{-3}$ M	$5 \times 10^{-3}$ M	$5 \times 10^{-3}$ M
minutes of induction				
7	3300	9100	11,700	6500
27	8100	33,100	34,600	23,900
47	10,300	55,500	47,200	36,200
67	10,200	67,800	70,500	43,300

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