FACTORS AFFECTING GROWTH INHIBITION OF ENTERIC BACTERIA BY METHYL α-D-GLUCOSIDE

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INTRODUCTION

In previous work done by D.J. Schnell and colleagues (1982) it was reported that a marine enteric bacterium, *Vibrio parahaemolyticus*, responded in a peculiar fashion to analogs of glucose. When methyl α-D-glucoside (αMG) was added to cultures of the organism growing on either mannose or fructose, there was an initial period of inhibition, two to four hrs in low phosphate media (0.6M), which was rapidly overcome and subsequent growth was no longer affected by αMG. This phenomenon has since been termed an "escape" from inhibition. Also, this work raised the question as to whether this "escape" differed from that observed in *E. coli*. (Figure 1).

The purpose of our experimental work this summer was to determine the escape behavior in *E. coli*, and to describe the mechanism of escape from the inhibition of αMG. In our studies this year, we decided to work with the more extreme halophilic marine bacterium *Vibrio harveyi* rather the *Vibrio parahaemolyticus*.

METHODS

Vibrio harveyi cells were grown on AK/0.4M NaCl media containing 10 mM of a carbon source, such as glucose, fructose, or mannose. For strains of *E. coli*, basal medium (BM) plus essential amino acids (tHALT) was used, as well as 10 mM of a carbon source. When analyzing the escape phenomenon, 2 mM αMG was added to the above media. For growth, all cultures were incubated at 30°C and left to shake. 2 mM [^{14}C] glucose, mannose, and αMG were used in our uptake studies. The final strength of the isotopes was 6 µCi/ml when the assay was performed with cells at an optical density of 0.15. Also sampling was done over a ten min period at two min intervals beginning one min after the addition of the isotope. To determine the phosphotransferase activity of these organisms, a technique developed by Kornberg and Reeves (1976), with slight modifications, was used. In this cells were harvested via centrifugation and then resuspended in PT/0.4M NaCl to an optical density of 2.0. The cells were then toluenized with a 10% solution of toluene and ethanol for exactly one min on the vortex mixer. From this point on, the method of Kornberg and Reeves was followed. The modifications were necessary because the cells require a salt concentration to lower than 0.08M. Without this the *Vibrios* will lyse due to an increased osmotic pressure. It should also be noted that too high a concentration of salt will hamper the phosphotransferase activity in the cells. Thus, a final salt concentration of 0.08M is the optimum condition for the phosphotransferase assay. The toluenization of the cells renders them permeable and through the addition of PEP, lactate dehydrogenase (LDH), reduced nicotinamide adenine denucleotide ($NADH_2^+$), and a carbon source; the phosphorylation of the carbon source can be measured directly by recording the decrease in optical density of $NIDH_2^+$ at 340nm (Figure 4).

DISCUSSION AND RESULTS

Strains of E. coli that are constitutive for the uptake of αMG did not exhibit the escape phenomenon, but that they are continually inhibited by the αMG. However, E. coli strains that are inducible for the MG uptake system, like the Vibrios, did escape from the inhibition of αMG. Moreover, cells must be pre-grown on glucose if escape is to be observed in the inducible strains (Figure 2). Therefore, it became clear that the extent and duration of inhibiton was dependent on the level of the αMG uptake system in the cell and the amount of αMG that was retained in the cell. To demonstrate this, we performed an experiment in which cells were grown on mannose and then transferred into mannose and αMG. We saw no inhibition of growth in these cells and found that the initial level of αMG in these cells was 10 nmoles, and this level decreased rapidly as growth began. However, then cells were grown on glucose and then transferred into mannose and αMG, there was a one hr inhibiton of growth and this was followed by an escape from this inhibition. In these cells, we found that the initial level of αMG was 28 nmoles and that this level decreased at a very slow rate until a low enough level was reached were growth could begin (Figure 3).

We then tested the hypothesis that this escape might be mediated by an alkaline phosphatase. From the work of Dr. Annemaria Torriani, growth in low phosphate media leads to the induction of phosphatases which, conceivably, could break down the αMG-phosphate accumulated in the cell and thus cause an escape from inhibition. To test this, wo obtained a mutant of E. coli in which the gene for alkaline phosphatase, phoA, was deleted. It behaved just like the inducible strain of E. coli shown in Figure 2, there was an initial inhibition and then escape. Therefore, we concluded that this phenomenon was not mediated by the derepression of the phoA gene.

The possibility then arose that this escape phenomenon might be due to the destruction of the αMG uptake system, when cells had been grown in the presence of αMG. We developed an assay for measuring the phosphotransferase activity in cells of V. harveyi that had been toluenized and this has been previously described in this paper under the METHODS section. The cells were pregrown on glucose and then transferred into either mannose alone or mannose and αMG, and they were allowed to go through three doublings before they were harvested for this assay. The loss of uptake activity for glucose and αMG were identical irrespective of whether there was αMG present in the growth medium or not, and were roughly what would be expected for "diluting out" of the activities intitially present (Figure 4, table 1).

However, the ability of whole cells to take up [^{14}C] labelled αMG did not reflect these data. Whereas cells harvested from mannose alone still took up [^{14}C] αMG, cells harvested from mannose and αMG completely failed to do so over the usual ten min sampling period (Figure 5). Since those same cells, when toluenized, still contain the phosphotransferase activity for αMG, the apparent failure to take up αMG might be due to an increased loss of the αMG from the cells, once it had accumulated. As the graph on Figure 6 shows, there is an initial uptake of labelled αMG which is then followed by a rapid loss of αMG; however, this did not occur in cells that had not been exposed to αMG during growth.

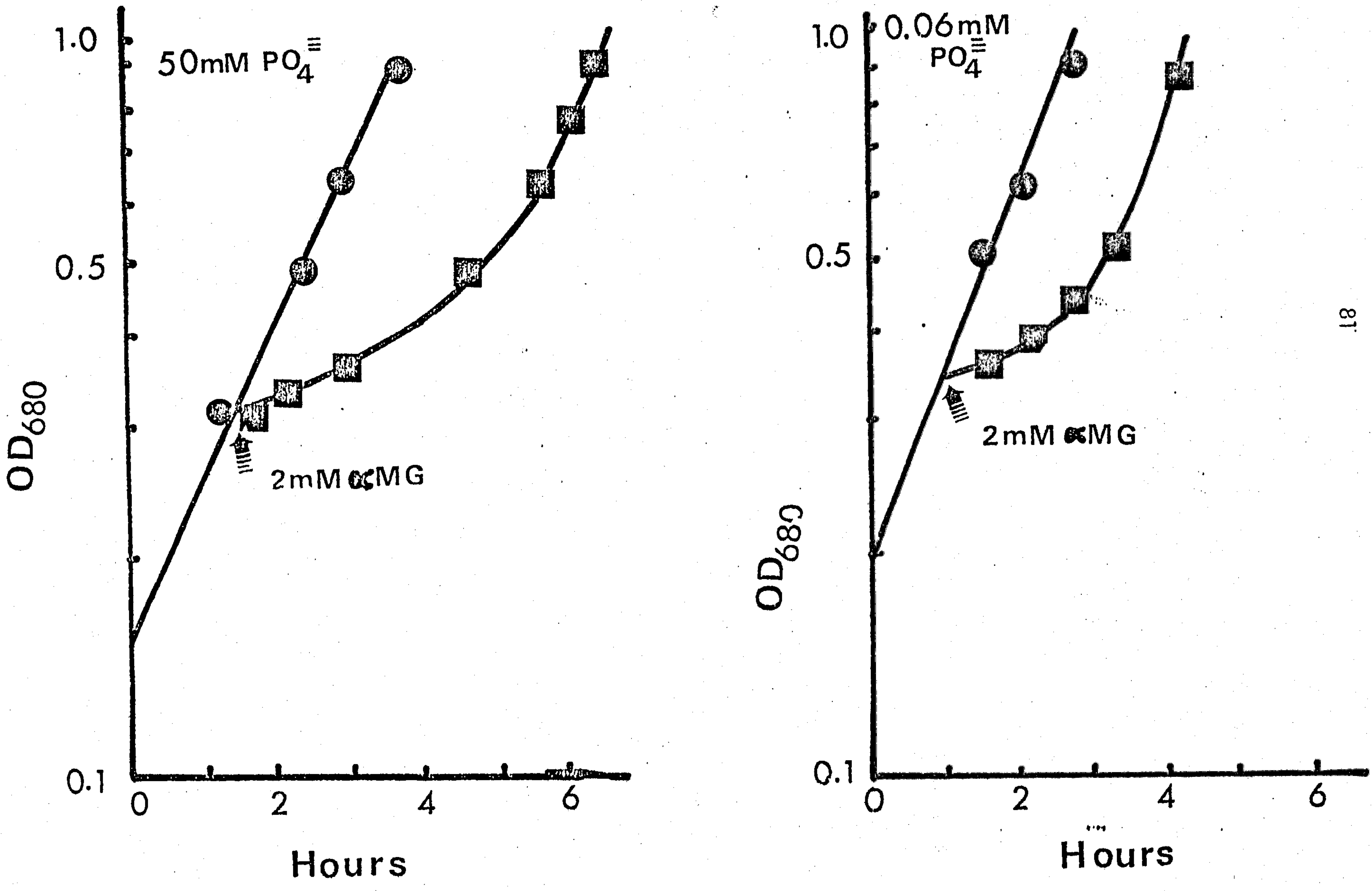
This activity, that presumably promotes the loss of αMG by somehow removing phosphate from the αMG-phosphate accumulated, could be due to a transphosphorylation in which the phosphate could be transferred from the internal αMG-phosphate to an external sugar, or to a novel intracellular, but not periplasmic, phosphatase. Since the inhibition and escape we saw with sugars taken up by phosphotransferase action also occurs with glycerol and cellobiose as carbon sources, which are not taken up by the PT-system, it is not likely to be a transphosphorylation reaction. But whatever new activity is elicited by αMG in the medium, it is likely to involve _de novo_ protein synthesis. If we grow cells in mannose alone and then transfer the cells to mannose, αMG, and chloramphenicol (100 μg/ml) for the same length of time as it takes cells, without chloramphenicol, to escape, we note that the cells still have the ability to take up [^{14}C] labelled αMG (Figure 6). Thus, the ability of cells to escape from the inhibiton of αMG is due to the _de novo_ synthesis of a protein that dephosphorylates the αMG already accumulated inthe cell (Figure 5). However, work still needs to be done to characterize this new protein and to determine its exact mode of regulation in strains of _Vibrio harveyi_ and in inducible strains of _E. coli_.

Table 1: Data on the phosphotransferase activity in cells grown in mannose or mannose and αMG.

PT-ACTIVITIES

	Cells Grown on:	
	Man	Man + αMG
Man	11 nmoles/min/ml	10
Glc	2	2
αMG	2	2

Figure 1: The escape from inhibition by αMG as exhibited by _V. parahaemolyticus_



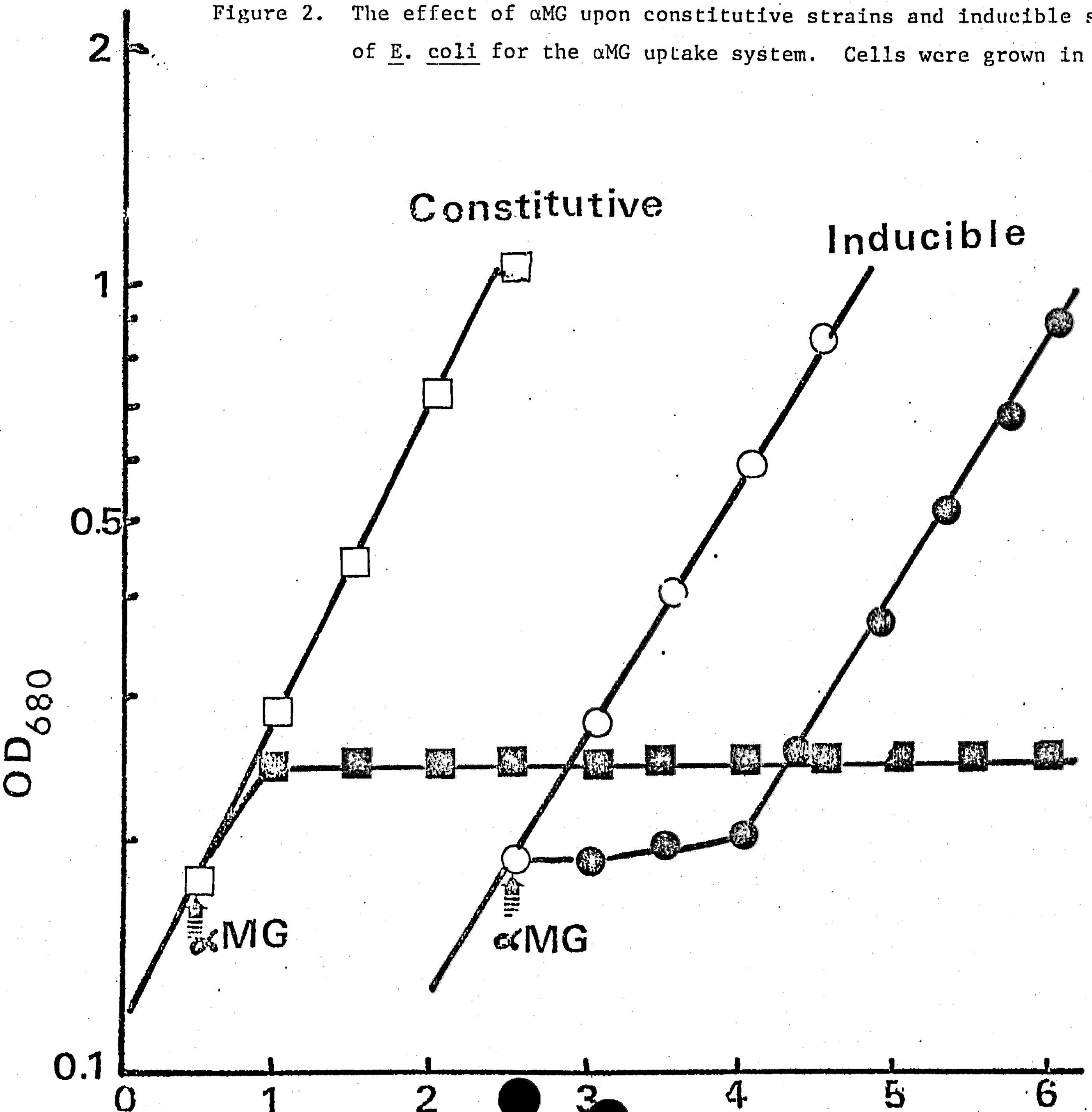


Figure 2. The effect of αMG upon constitutive strains and inducible strains of *E. coli* for the αMG uptake system. Cells were grown in mannose.

Figure 3. The retention of αMG by cells pre-grown on mannose and pre-grown on glucose

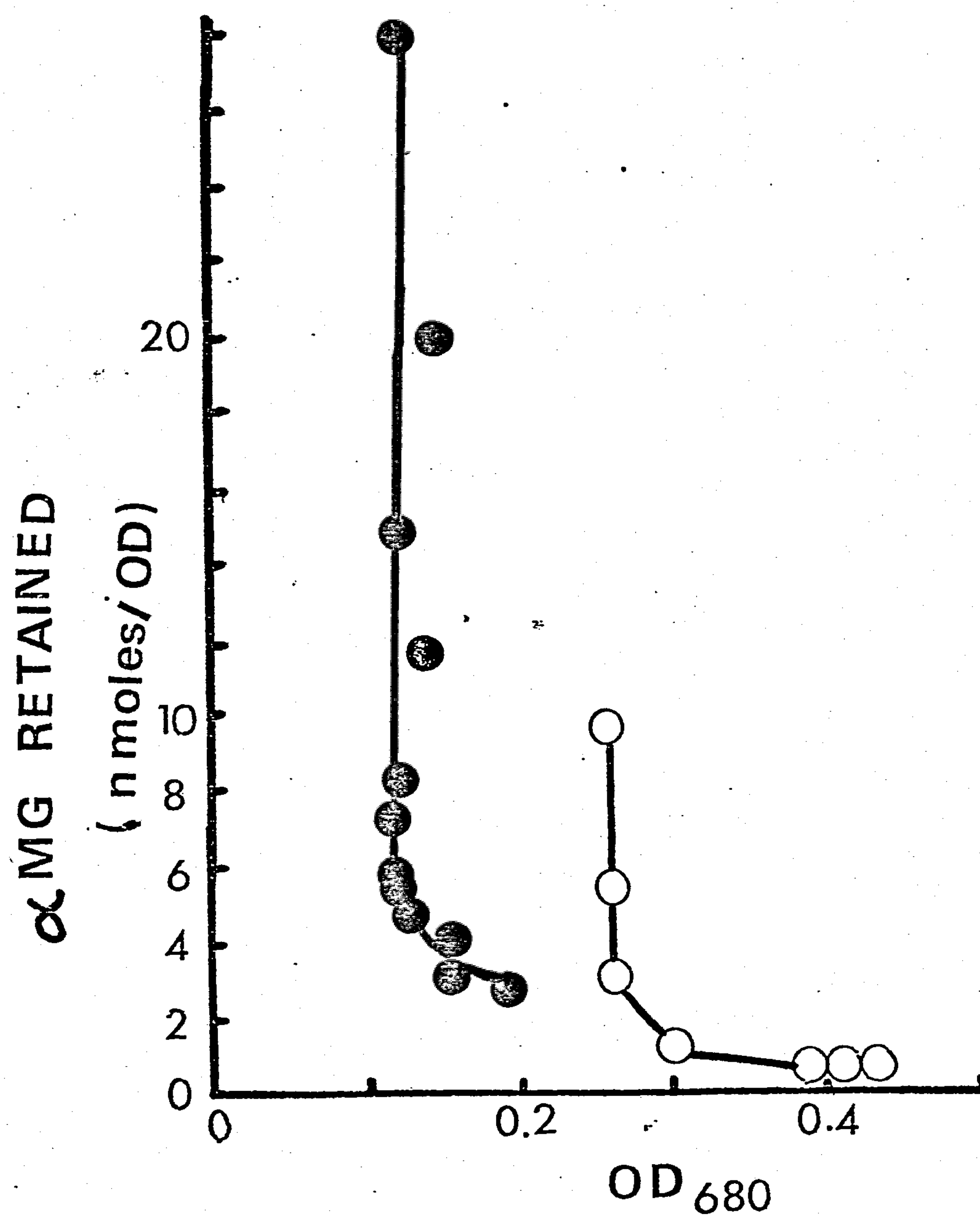


Figure 4. Diagram of the basis for the assay on phosphotransferase activity of toluenized cells

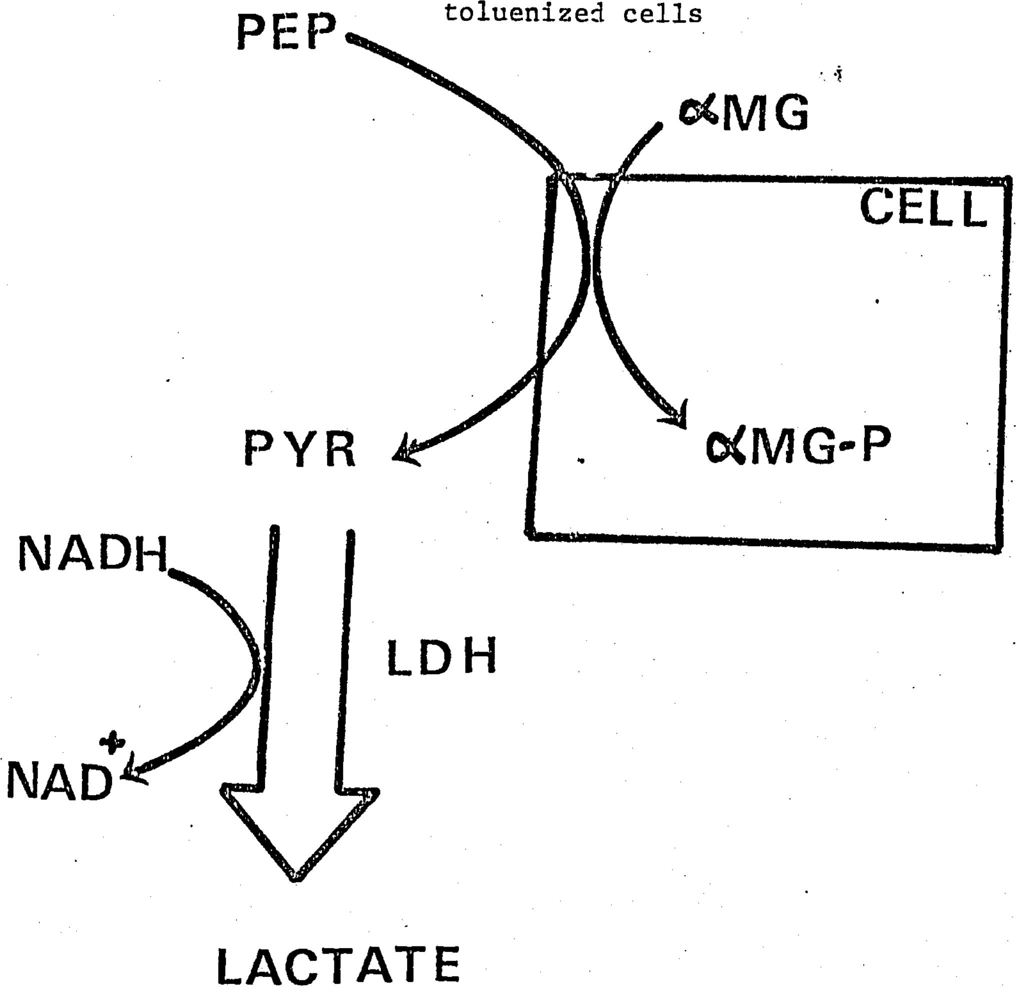


Figure 5. Uptakes of [^{14}C] labelled mannose and αMG by cells grown in mannose + αMG + chloramphenicol (100 μg/ml), or mannose + αMG

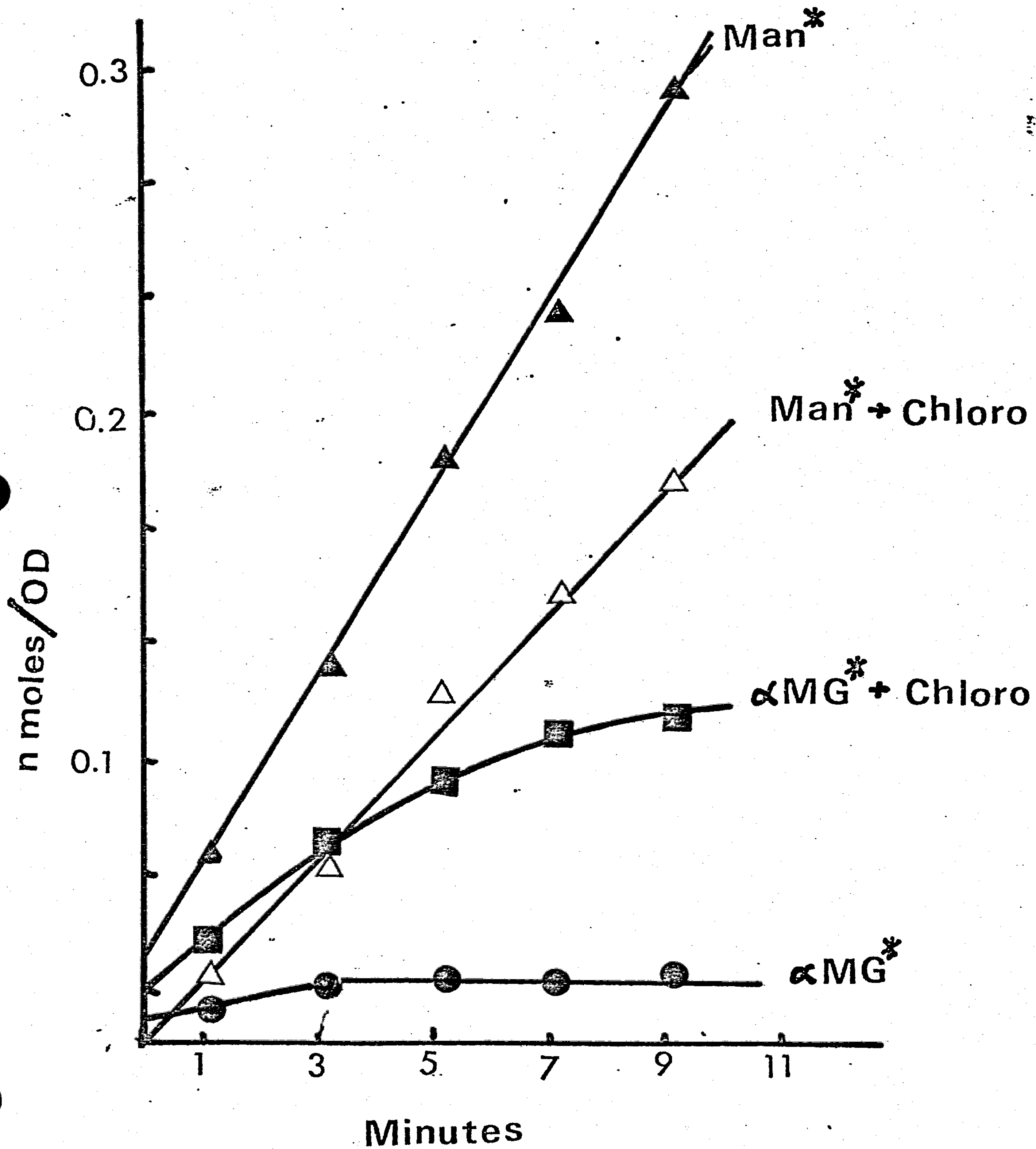


Figure 6. Uptake of αMG cells incubated with chloramphenicol, or without chloramphenicol over a four minute sampling period. All cells grown on Mannose + αMG