The Reversal of Glucose Repressed Prodigiosin Production in *Serratia marcescens* by the Cyclic 3′5′-Adenosine Monophosphate Inhibitor Theophylline

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Summary. Glucose was found to cause severe repression of prodigiosin production in *Serratia marcescens* and a dose related partial reversal was demonstrated by theophylline. It is suggested that this reversal is due to the inhibition of cAMP phosphodiesterase and the concomitant increase in cellular cAMP concentration.

It has been reported[1] that a cyclic 3′5′ adenosine monophosphate phosphodiesterase (PDE) from *Serratia marcescens* was competitively inhibited by theophylline, in contrast to that of later work[2] which reported the lack of inhibition of a comparative enzyme from a strain of *Escherichia coli*. Several workers[3] have also demonstrated the glucose repression of prodigiosin synthesis in strains of *Serratia marcescens* and a recent review[4] reports the cAMP involvement in prodigiosin synthesis. It was the intention of this study to establish whether in vivo inhibition of this enzyme would result in a demonstrable reversal of glucose repression of prodigiosin synthesis.

Materials and methods. The test strain of *Serratia marcescens* was kindly supplied by Dr. A. G. Schaffer of Bristol Polytechnic. Theophylline was obtained from B. D. H. Chemicals Ltd.

An overnight culture was grown in the following medium, which is referred to as proliferation medium, at 37 °C and 100 rpm in a Gallenkamp orbital incubator shaker. KH_2PO_4 4.5, K_2HPO_4 10.5, $(NH_4)_2SO_4$ 1.0, $MgSO_4.7H_2O$ 0.25, sodium citrate 0.5, yeast extract 5.0, vitamin free Casamino acids 5.0 (all values quoted as g/l). 100 ml in a 1 l conical flask. This culture was used to inoculate, at 1%, 6 × 100 ml volumes of proliferation medium and further incubated overnight at the same incubation conditions supra vide. In this medium, at these incubation conditions, no pigment was formed. The growth was harvested by centrifugation, washed once with phosphate buffer containing KH_2PO_4 4.5, and K_2HPO_4 10.5 g/l, and resuspended in half the volume of production medium containing L-proline 5.0, K_2HPO_4 10.5, and KH_2PO_4 4.5 g/l. The absorbance at 600 nm (OD_{600}) was measured (1 cm cell) with a Pye Unicam S.P. 600 spectrophotometer, and 20 ml aliquots were dispensed in 250 ml conical flasks. 1 ml additions were made as filter sterilized aqueous soutions as follows: glucose 11%, theophylline 44, 22 and 11 m*M*. Volumes were made up, where necessary, to 22 ml with sterile distilled water. The flasks were incubated for 48 h at 30 °C and 120 rpm. At this time, the cultures were monitored for OD_{600} and extracted for assay of the prodigiosin content.

To 10 ml volumes of culture were added 0.5 ml 2 *N* HCl and 0.5 ml 10% formal saline. The resultant mixtures were shaken with 10 ml volumes of butan-2-one and the emulsions were centrifuged to separate the organic layers. These were retained for the measurement of the absorbance at 540 nm (OD_{540}) (1 cm cell). After this extraction the cells were visually devoid of pigment. The content of prodigiosin in the extracts was calculated with reference to the molar extinction coefficient of prodigiosin HCl at 540 nm. $E = 7.07 \times 10^4$ (ex. Merck Index).

Results. The results of a typical experiment are depicted in the Table. The specific content of prodigiosin is (the ratio of prodigiosin content per ml of extract to the OD_{600} value) × 100. The Figure depicts the concentration of theophylline plotted against average specific prodigiosin content. The glucose repression of prodigiosin production was found to be partially relieved by theophylline in a dose related manner. 2 m*M* theophylline increased the specific content of prodigiosin, in glucose containing media, by a factor of approximately 2.3.

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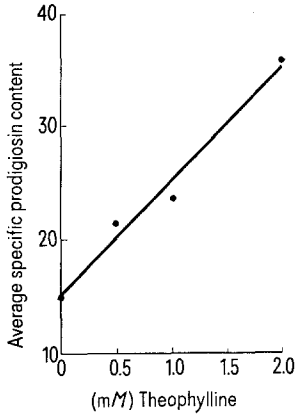
The effect of theophylline on the glucose repression of prodigiosin synthesis

	Initial OD_{600}	Final OD_{600}	OD_{540}	Prodigiosin (μg/ml)	Prodigiosin specific content	Average
No addition	3.47	4.84	2.0	10.2	211	205.0
No addition	3.47	4.57	1.8	9.1	199	
0.5% glucose	3.47	6.02	0.184	0.9	15	15.0
0.5% glucose	3.47	6.01	0.174	0.9	15	
0.5% glucose + 2 m*M* theophylline	3.47	5.48	0.345	1.8	33	36.0
0.5% glucose + 2 m*M* theophylline	3.47	5.45	0.410	2.1	39	
0.5% glucose + 1 m*M* theophylline	3.47	6.01	0.280	1.4	23	23.5
0.5% glucose + 1 m*M* theophylline	3.47	5.80	0.273	1.4	24	
0.5% glucose + 0.5 m*M* theophylline	3.47	5.58	0.238	1.2	22	21.5
0.5% glucose + 0.5 m*M* theophylline	3.47	5.69	0.232	1.2	21	

Washed cells suspended in production medium, containing L-proline 5.0 g/l, K_2HPO_4 10.5 g/l and KH_2PO_4 4.5 g/l plus additions and after 48 h incubation, at 30 °C, monitored for OD_{600} and prodigiosin content of extracts by measurement of OD_{540}. OD_{600} values obtained from adjusted values of 1/10 dilutions.

Discussion. It is postulated that the reversal of the glucose repression of prodigiosin synthesis by theophylline is the result of the inhibition of PDE and the concomitant increase in cellular cAMP concentration. However, further studies, involving the measurement of cellular cAMP levels, are required to substantiate the validity of this suggestion.

With reference to the studies of β-galactosidase induction and repression in catabolite repression sensitive and insensitive strains of *E. coli*[5], and their cAMP degradative abilities[2,6] it was not expected to achieve complete reversal of glucose repression of prodigiosin production and this was not achieved with the doses of theophylline utilized. However, 2 m*M* theophylline did cause approximately a doubling of specific content.



The concentration of theophylline plotted against the average specific prodigiosin content for glucose repressed cells of *S. marcescens*.

It is interesting to note that the reversal of glucose repressed motility in *E. coli* by theophylline has been reported[7], which ,if one assumes its action to be via PDE inhibition, is at variance with the in vitro *E. coli* inhibition study. Un reported work of mine failed to demonstrate theophylline reversal of glucose repressed β-galactosidase induction in *E. coli* K12, but this, however, could be due to the insensitivity of the enzyme, or to the inherent differing sensitivities of the different systems to the modulation of cAMP levels.

It is suggested that this work may form the basis for the development of a correlative screen for potential pharmacologically active compounds, the mode of action of which are, in part, or in total, the inhibition of PDE.

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Identification of Uncommon Amino Acids in the Lentil Seed (*Lens culinaris Med.*)

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Summary. The fraction of the free basic amino acids in the lentil seed was shown to contain γ-hydroxyarginine, γ-hydroxyornithine and homoarginine besides the common amino acids. Similar distribution was found in 5 varieties of lentil, with hydroxyarginine and arginine dominating. The significance of these findings with regard to chemotaxonomy and lentil consumption is discussed.

In a previous report, γ-hydroxyarginine and γ-hydroxyornithine were shown to occur as free amino acids in 5 varieties of lentil seeds[1]. These compounds were identified by comparison with synthetic samples on an amino acid analyser, under different elution conditions. As the native compounds from lentil are stereo-chemically uniform, and since chemical synthesis of each of these hydroxylated amino acids yields a mixture of the two possible diastereomeric forms, full elucidation of their structures could not be accomplished[2]. For further study, γ-hydroxyarginine, representing up to 70% of the total free basic amino acids in lentil seeds, was isolated from a seed extract by ion exchange chromatography[3]. An unidentified compound, showing a ninhydrine-positive reaction and eluted after arginine, was further isolated by the same separation procedure[4].

Treatment by 6 *N* hydrochloric acid, under reflux, left the compound unchanged. Boiling for 1 h with 2 *N* sodium hydroxide decomposed the sample to lysine, urea and decomposition products of the latter, in equimolar ratio. The presumption that it was homoarginine could be made by comparison with an authentic sample of homoarginine (mixed samples, using buffers of different pH values in the analyser).

The Table shows the distribution of the free basic amino acids in 5 varieties of lentils investigated[5]. Hydroxyarginine and arginine dominate over hydroxyornithine and the common basic amino acids. On the basis of these findings, homoarginine, as well as hydroxyarginine and hydroxyornithine, has to be considered a regular and specific component of the lentil seed. The occurrence of uncommon metabolites may be helpful to botanists for classification work, or for the study of phylogenetic relationships. The plant family of Leguminosae offers a great field of chemotaxonomic study. It should be men-

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[2] H. SULSER and F. SAGER, Lebensm.-Wiss. + Technol. *7*, 327 (1974).

[3] H. SULSER, M. BEYELER and F. SAGER, Lebensm.-Wiss. + Technol., *8*, 161 (1975).

[4] For experimental details concerning ion exchange chromatography and analysis of fractions, see ref.[3]. Homoarginine was collected between 10 and 12 l of eluant.

[5] The determination of the hydroxy amino acids is sensitive to variation, since these compounds are partially converted to γ-lactones, even under slightly acidic conditions. The concentrations given in the Table have, therefore, to be regarded as approximate values.