APPENDIX

**Two Separate Genes Involved in Sulphate Transport in
Escherichia coli K12**

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INTRODUCTION

In the course of the experiments described above it became clear that the two *cys* mutations used were not identical. The *cysA471* mutation was isolated by Tully (1976) as conferring resistance to chromate, the *cysZ474* mutation arose spontaneously and simultaneously with a *ptsI* mutation, (it may be the result of an inversion with ends in these two genes). The mapping of the genes (see above, Table 3) shows that *cysA* and *cysZ* lie on opposite sides of *ptsI*. We therefore investigated the biochemical nature of the mutations.

METHODS

Exponential phase cells growing in minimal medium supplemented with cystine were harvested, resuspended in the sulphur-free medium of Pasternak (1962) with 10 mM-gluconate as carbon source and incubated at 37 °C for 9 h to derepress the sulphate transport system. The cells were harvested and resuspended in 5 mM-HEPES buffer, pH 6.5, at a concentration of 0.68 mg dry mass ml⁻¹. Radioactive sulphate [³⁵SO₄²⁻] was added to a final concentration of 0.2 mM and 20 µCi ml⁻¹, and the cells were incubated at room temperature. Samples were taken at 10, 60, 120 and 180 s, filtered through an Oxoid membrane filter and washed four times with 2 ml HEPES buffer. The filters were dissolved in 10 ml of scintillation fluid [0.4% 2,5-diphenyloxazole, 80% (v/v) toluene and 20% (v/v) methoxyethanol] and their radioactivity was measured in a Packard 3385 scintillation spectrometer. Background measurements were made under identical conditions and samples of [³⁵SO₄²⁻] were assayed to convert the disintegrations measured to moles of sulphate ions. All other techniques were as described in the main paper.

RESULTS

Sulphate transport was assayed in the cysteine auxotrophs JM1783 (*cysA*) and JM1766 (*cysZ*) and in isogenic wild-type strains (Table 5). These strains were also tested for their growth response to thiosulphate at 20 mM, a concentration that supports the growth of mutants of *Salmonella typhimurium* LT2 defective in sulphate transport (Mizobuchi *et al.*, 1962). The results are shown in Table 5. The strains are deficient in sulphate accumulation, but apparently not in the synthesis of cysteine from *O*-acetylserine and sulphide, since they grow when supplemented with 20 mM-S₂O₃²⁻.

DISCUSSION

In *Salmonella typhimurium* LT2 three closely-linked cistrons *cysAa*, *Ab* and *Ac* are required for sulphate transport (Howarth, 1958; Mizobuchi *et al.*, 1962; Ohta *et al.*, 1971), though none of these is the structural gene for the sulphate binding protein. A deletion of all three cistrons (Howarth, 1958) was not reported as being deficient in glucose utilization, suggesting that the known *Salmonella cysA* cistrons do not bracket *ptsH* and *I*. Karbonowska *et al.* (1977) indicate that the *cysA* chromate-resistant mutations of *E. coli* and *S. typhimurium* are probably in corresponding cistrons. If this is so, the *ptsH* and *ptsI* genes of *Salmonella* are inverted relative to *cysA*.

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Table 5. *Properties of the sulphate transport deficient mutants*

Sulphate transport was measured after 9 h of sulphur starvation as described in Methods.

Strain	Pertinent genotype	Growth		Sulphate transport [pmol min ⁻¹ (mg dry mass) ⁻¹]
		-S ₂ O ₃ ²⁻	+S ₂ O ₃ ²⁻	
JM1783	<i>cys</i> ⁺	+	+	260
JM1781	<i>cysA471</i>	-	+	40
CBK110	<i>cys</i> ⁺	+	+	275
JM1766	<i>cysZ474</i>	-	+	65

We have not assayed our *cys* mutants for any of the enzymes of cysteine biosynthesis, most of the structural genes for which map elsewhere (Jones-Mortimer, 1968, 1973) but the growth of the mutants on medium supplemented with 20 mM-thiosulphate indicates that at least one of the isoenzymes of *O*-acetylserine sulphydrase (Kredich *et al.*, 1980) is present in the strains. In *Salmonella* the genes for both isoenzymes map near *ptsI* (Kredich *et al.*, 1980). Wiater & Hulanicka (1979) demonstrated a deficiency in sulphate transport in *cysK* mutants of *E. coli*. These, like the *cysK* mutants of *S. typhimurium* are defective in *O*-acetylserine sulphydrase-A (Hulanicka *et al.*, 1975). Our (unpublished) experiments indicate that the *cysZ* gene cannot be identical with *cysK*, since a plasmid carrying the *cysK*⁺ gene did not complement the *cysZ* defect.

Since SO₄²⁻ ions that enter the cells can presumably be metabolized in the mutants as well as in the wild-type strains, the observed rates of assimilation must represent the sum of transport and metabolism.

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