

## EVIDENCE FOR A FOUR-SULPHUR INTERMEDIATE IN THIOSULPHATE OXIDATION BY *THIOBACILLUS X*\*

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The autotrophic bacteria, *Thiobacillus* spp., oxidize thiosulphate to sulphate but the mechanism of this reaction is obscure (see Vishniac and Trudinger 1962). The suggestion that tetrathionate is an intermediate (Tamiya, Haga, and Huzisige 1941; Vishniac and Santer 1957; Jones and Happold 1961) has been contested by Ostrowski and Krawczyk (1957) and by Peck (1960), who have proposed that a scission of the thiosulphate molecule takes place prior to oxidation.

Tetrathionate accumulates under some conditions during the oxidation of thiosulphate by thiobacilli [see reviews by Vishniac and Santer (1957) and by Trudinger (1959)] but it has been pointed out by Lees (1960) and Vishniac and Trudinger (1962) that this may not necessarily implicate tetrathionate as an intermediate in thiosulphate oxidation.

In this paper an experiment is described which provides further evidence that in the early stages of thiosulphate oxidation by the aerobic sulphur autotroph, *Thiobacillus X*, a four-sulphur intermediate is formed.

### *Experimental Procedure*

Washed cells of *Thiobacillus X* (7.2 mg dry wt.) prepared as described earlier (Trudinger 1961a) were incubated in Warburg flasks at 30°C with 200  $\mu$ moles of potassium phosphate, pH 7, and 32  $\mu$ moles of  $\text{Na}_2\text{S}_2\text{O}_3$ , labelled with  $^{35}\text{S}$  in either the inner ( $-\text{SO}_3$ ) or outer (S-) position (New England Nuclear Corporation, Boston, Mass.) in a volume of 2 ml. At various times the reactions were stopped by the addition of 2 ml 10% (v/v) acetic acid in 50% (v/v) ethanol and the mixtures diluted to 10 ml with water and centrifuged. Sulphate was separated from the supernatants by chromatography on Dowex 1X2 (Trudinger 1964a) and  $^{35}\text{S}$  measured by plating aliquots on stainless steel planchets (Trudinger 1961b) and counting in a gas-flow counter (Nuclear-Chicago model D47).

The precipitates were washed twice with 0.1M potassium phosphate, pH 7, and oxidized with a mixture of HCl,  $\text{HNO}_3$ , and  $\text{Br}_2$  for the determination of  $^{35}\text{S}$  (Trudinger 1964b). This material is referred to as precipitated sulphur.

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### Results

Figure 1 shows the course of sulphate and precipitated sulphur production from the inner and outer groups of thiosulphate during the initial stages of thio-sulphate oxidation by *Thiobacillus X*. For the first 12–13 min, when about 17% of the oxygen required for complete oxidation had been consumed, approximately one molecule of sulphate and one atom of precipitated sulphur were formed from the outer group for every two molecules of sulphate derived from the inner sulphur.

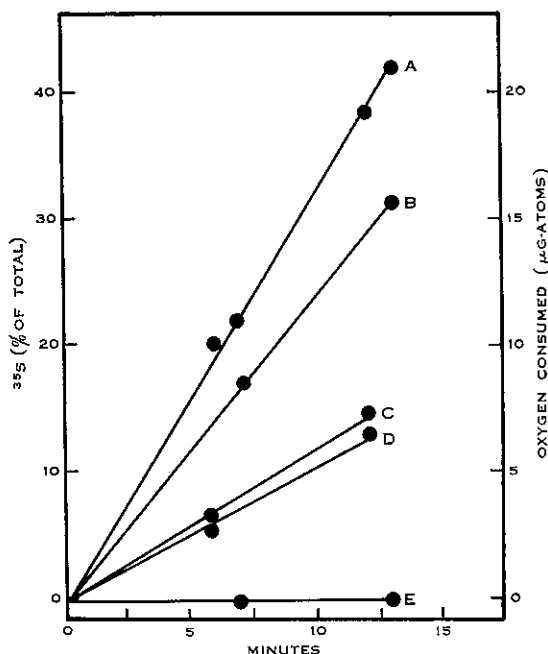


Fig. 1.—Production of sulphur and sulphate from [ $^{35}\text{S}$ ]thiosulphate. For experimental conditions see text. A, oxygen consumption ( $\mu\text{g-atoms}$ ). B,  $^{35}\text{S}$  in sulphate from inner-labelled  $\text{Na}_2\text{S}_2\text{O}_3$  (specific activity  $85.6 \times 10^3$  counts/min/ $\mu\text{mole}$ ); slope 2.50. C,  $^{35}\text{S}$  in sulphate from outer-labelled  $\text{Na}_2\text{S}_2\text{O}_3$  (specific activity  $188 \times 10^3$  counts/min/ $\mu\text{mole}$ ); slope 1.26. D,  $^{35}\text{S}$  in precipitated sulphur from outer-labelled  $\text{Na}_2\text{S}_2\text{O}_3$ ; slope 1.20. E,  $^{35}\text{S}$  in precipitated sulphur from inner-labelled  $\text{Na}_2\text{S}_2\text{O}_3$ ; slope 0.

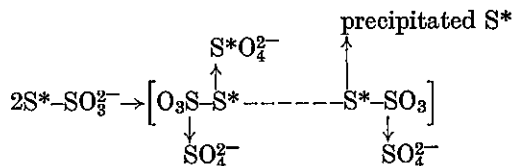
The oxidation of the precipitated sulphur to sulphate by a fresh sample of *Thiobacillus X* required approximately three atoms of oxygen per atom of sulphur, indicating that this material was largely at the oxidation level of elementary sulphur.

Qualitative examination of the supernatants by paper electrophoresis (Trudinger 1961b) showed that, apart from sulphate and precipitated sulphur, only tetrathionate and traces of trithionate were formed from thiosulphate (cf. Trudinger 1959).

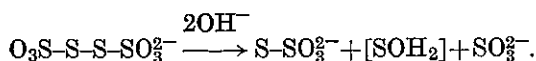
### Discussion

The quantitative relationships between precipitated sulphur and sulphate formed from the inner and outer groups of thiosulphate suggest that these compounds

were derived from the breakdown of a four-sulphur intermediate according to the following scheme:



Although tetrathionate is formed during the oxidation of thiosulphate by *Thiobacillus X*, recent evidence (Trudinger 1964a) indicates that its subsequent metabolism involves an initial breakdown to thiosulphate according to the equation



Tetrathionate, therefore, does not appear to fit the requirements for the postulated four-sulphur intermediate in thiosulphate oxidation and is more likely to have arisen from the intermediate by a secondary reaction (cf. Lees 1960; Vishniac and Trudinger 1962).

The proposed reaction scheme differs from those put forward by Ostrowski and Krawczyk (1957) and Peck (1960). Ostrowski and Krawczyk, however, based their arguments on the results of long-term growth experiments which give little indication of the initial steps in thiosulphate oxidation, while Peck's conclusions were based on experiments with cell-free extracts in the presence of substrate amounts of glutathione which was required for oxidative activity. It is quite possible, in the latter case, that some disorganization of the reaction sequence resulted during extraction of the cells. Furthermore thiols and disulphides react readily with thiosulphate and other inorganic sulphur compounds (e.g. Parker and Kharasch 1959) and some modification of the metabolic pathway may well have been introduced by the presence of excess glutathione.

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