both of which are known to contain acid mucopolysaccharides.[11] Since the rate of synthesis of mucopolysaccharides in embryonic tissues may play a role in their growth and differentiation,[12] a relationship may be inferred between the known teratogenic action of drugs such as salicylate [4-6] and cortisone [3] and their inhibitory effect on mucopolysaccharide synthesis as evidenced either by depression of sulfate incorporation or, as presented in this report, by depression of the synthesis of a key intermediate in mucopolysaccharide biosynthesis.

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Mechanism of Oxidation of Inorganic Thiosulfate and Thiosulfate Esters in Mammals

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Thiosulfate is a metabolic product in higher animals [1] and is used as antidote against cyanide,[2] sulfur and nitrogen mustards.[3,4] A knowledge of its metabolism in higher animals is consequently of interest. Early investigators [5] demonstrated that thiosulfate injected into mammals is oxidized to sulfate, and Pirie [6] found that slices from certain rat tissues could accomplish this reaction although extracts from these tissues were practically inactive. Using thiosulfate labelled with ^{35}S in the outer and inner position, Skarzynski *et al.*[7] found that when thiosulfate was injected into rats most of the sulfate formed was derived from the inner sulfur atom, whereas the outer sulfur atom entered into tissue metabolism. No further details about the mechanism of thiosulfate oxidation in higher animals are available. However, certain microorganisms, for instance the sulfur bacteria, can also oxidize thiosulfate to sulfate, and the enzymatic mechanisms responsible for this reaction in *Thiobacillus thio-oxidans* have recently been elucidated by Peck.[8] In this case, thiosulfate is first reduced to sulfite by glutathione in a reaction catalyzed by the enzyme thiosulfate-reductase which was previously discovered in yeast by Kaji and McElroy.[9] The sulfite thus formed is then oxidized to sulfate in an AMP and phosphate dependent reaction. The aim of the present investigation was to study if the oxidation of thiosulfate to sulfate in rat liver takes place by the same mechanism as in bacteria.

The ability of rat liver slices to form sulfate from thiosulfate in an oxygen atmosphere as reported by Pirie [6] was first confirmed in the present investigation. It was necessary to carry out the reaction in oxygen, as otherwise the small amounts of sulfate found were barely detectable by the analytic technique used. An average value of 84.5 $\pm$ 10.2 (mean $\pm$ standard error of 8 determinations) μmoles of sulfate

formed per hour per g dry weight was obtained, and this value is of the same magnitude as that found under similar conditions by Pirie. In confirmation of Pirie's results it was also found that homogenisation destroyed most of the activity. However, if a rat liver homogenate was fortified with glutathione or certain other mercaptans (Table 1), a stimulation

Table 1. Effect of thiols on oxidation of thiosulfate by liver homogenates.

The reaction mixture contained 4 μmoles $Na_2S_2O_3$, 4 μmoles thiol compounds where indicated, 62 μmoles $NaHCO_3$ and 100 mg rat liver in a final volume of 2 ml. This was incubated 30 min at 37° in Warburg flasks in 95 % O_2 – 5 % CO_2. Sulfate was then determined with barium chloroanilate.[16]

Addition	*Sulfate formed* μmoles
None	0.19
Glutathione	1.47
Dihydrolipoate	1.48
Cysteine	0.46
Mercaptoethanol	0.93

of sulfate formation occurred. This indicates a participation of thiosulfate reductase in the oxidation of thiosulfate in rat liver as in bacteria. However, the liver extracts were active without AMP and phosphate both of which were required in the *Thiobacillus* system. This is explained by the presence, in rat liver, of a sulfite oxidase [10] which is not dependent on these cofactors. The figures given in Table 1 for the activity obtained with liver extracts fortified with glutathione or dihydrolipoate, correspond to 146 μmoles of sulfate formed per hour per g dry weight of liver, and they satisfactorily explain the activity figures obtained with slices. The ability of other mercaptans to replace glutathione in the liver system (Table 1) may be related to the fact that thiosulfate reductase from yeast [9] does not show an absolute substrate specificity with respect to mercaptans. But the fact that thiosulfate oxidation in rat liver was stimulated by dihydrolipoate is of special interest, as it was recently reported [11] that the enzyme rhodanese catalyzed the reduction of thiosulfate to sulfite by dihydrolipoate. As rhodanese cannot use glutathione as a substrate,[11,12] these findings would suggest two possible pathways for the enzymatic reduction of thiosulfate in liver, one using glutathione (catalyzed by thiosulfate reductase) and another using dihydrolipoate (catalyzed by rhodanese). If thiosulfate oxidation in the intact tissue were dependent on dihydrolipoate, one would expect the reaction to be inhibited by arsenicals and the inhibited system to be reactivated by dithiols [13] but not by monothiols. If, on the other hand, thiosulfate oxidation were glutathione dependent, any inhibition with arsenicals should be reversed by monothiols as well. When such experiments were carried out with liver slices (Table 2),

Table 2. Inhibition of thiosulfate oxidation by arsenite and its reversal by thiols.

The experiments were carried out in Warburg flasks which contained 62 μmoles of $NaHCO_3$ and 100 mg liver slices in the main compartment together with 0.4 μmoles of arsenite. The inhibitor was preincubated with the slices for 20 min at 37°, whereupon the reactivator (BAL or mercaptoethanol) was added from a side arm and incubation was then continued for an additional 20 min. The enzymatic reaction was started by the addition of 4 μmoles of thiosulfate from another side arm and allowed to proceed for 60 min. The inhibitor and reactivator were omitted from the control. Other conditions were as in Table 1.

	Sulfate formed μmoles/g liver/h	% *Activity*
Control	88.7	100
Arsenite	25.9	29.3
BAL	82.7	93.2
Arsenite + BAL	52.7	59.6
Mercaptoethanol	77.7	87.8
Arsenite + mercaptoethanol	45.8	51.8

it was found that 10^{-4} M arsenite gave about 70 % inhibition which was partially reversed by thiols, a monothiol (mercaptoethanol) and a dithiol (BAL) being equally effective in this respect. These data thus indicates that the glutathione dependent enzyme is the most important in the oxidation of thiosulfate. The slight inhibitory action of the thiols themselves may be noted, and in the case of mercaptoethanol can be explained by the fact that this compound is a less active substrate for thiosulfate reductase than glutathione (*cf.* Table 1).

As thiosulfate reductase has so far only been reported to occur in microorganisms it was important to demonstrate its presence also in liver homogenates. The assay system previously used for this enzyme in yeast or bacterial extracts [8,9] had to be modified for use with liver homogenates, as the latter contained high amounts of haem proteins which interfered with the determination of the reaction products. With a modified test system, based on the determination of the sulfite formed after anaerobic incubation of the enzyme and substrates, it was possible to demonstrate the presence of thiosulfate reductase in liver homogenates (Table 3).

Table 3. Glutathione dependent reduction of thiosulfate and thiosulfate esters in rat liver homogenates.

The reaction mixture contained 100 μmoles of phosphate buffer pH 7.4, and 100 mg of rat liver where indicated, in a final volume of 2 ml. When thiosulfate was used as substrate, the test system contained 20 μmoles of this compound and 20 μmoles of glutathione and was incubated for 30 min at 37°C. When thiosulfate esters were used as substrates, the test system contained 2 μmoles of these and 2 μmoles of glutathione and was incubated for 2 min at 0°C. All incubations took place in purified nitrogen. Sulfite was determined as previously described.[15]

Substrate	*Enzyme*	*Sulfite formed* μmoles
Thiosulfate	−	0.015
»	+	0.384
Ethylthiosulfate	−	0
»	+	0.417
Aminoethylthiosulfuric acid	−	0.082
»	+	0.164

Thiosulfate esters were also reduced by this system (Table 3). This is of interest, as one of these esters, aminoethylthiosulfuric acid, is a radioprotective agent [14] and is oxidized to sulfate by rat liver homogenates.[15] Previous experiments [15] suggested that this oxidation occurred through a preliminary conversion to sulfite in the presence of glutathione, and a subsequent oxidation of the sulfite to sulfate. As the spontaneous reaction between the thiosulfate esters and glutathione was found to occur fairly rapidly under the conditions used, it was suggested that this reactoin also occurred spontaneously in the body. However, by lowering the substrate concentration and the reaction temperature (Table 3), it was possible to demonstrate the catalytical effect of a rat liver homogenate on this reaction. The oxidation of thiosulfate esters *in vivo* thus occurs in an analogous fashion to that of inorganic thiosulfate.

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