in the 2-week-old rats, ascorbic acid either performs additional functions or performs the same functions as in the other two groups but at accelerated rates. Since the bone marrow is predominantly engaged in the production of blood cells, it is possible that some relationship may exist between the rate of production of blood cells and the quantity of ascorbic acid available to the bone marrow.

The concentrations of ascorbic acid in the various organs show a general decrease with age. The liver, which is mainly responsible for its synthesis, also shows a lower concentration of ascorbic acid: this may be due to a decrease in the activities of the enzymes which synthesize it. This may result in lower concentrations in other organs also. The reason for its lower concentration in the liver of older guinea pigs and in the blood cells of older men (Mourhouse & Guerrant, 1952; Kirk & Chieffi, 1953) kept on a normal ascorbic acid diet is not known. Our studies on the rat show that this lower concentration in the organs may be due either to a decrease in its rate of synthesis in the liver or to a decreased rate of its uptake by the organs.

SUMMARY

1. Parallel experiments to determine the uptake of ascorbic acid by the skin and bone marrow and for the determination of its concentration in the skin, liver, brain and kidney were undertaken in 2-, 10- and 50-week-old rats to evaluate the role of ascorbic acid at different ages of the animal.

2. Uptake of ascorbic acid occurred in the skin of 2-week-old rats. There was no uptake by the skin of the other two groups. It was concluded that ascorbic acid is not required for the maintenance of collagen in the skin.

3. The bone marrow of all the three groups of rats showed uptake of ascorbic acid, but that in the 2-week-old rats was fivefold greater. It is suggested that there may be some relationship between the rate of production of blood cells by the bone marrow and the quantity of ascorbic acid available to it.

4. There was a general decrease with age of the concentration of ascorbic acid in various organs, including the liver. It is possible that there may be a decrease with age in the activities of the enzymes responsible for the synthesis of ascorbic acid in the liver.

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The Effects of Thiosulphate and Oxygen Concentration on Tetrathionate Oxidation by *Thiobacillus X* and *T. thioparus*

By P. A. TRUDINGER*

Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T., Australia, and Department of Biology, Haverford College, Haverford, Pa., U.S.A.

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Tetrathionate is readily oxidized to sulphate by a number of aerobic and anaerobic thiobacilli (Baalsrud & Baalsrud, 1954; Parker & Prisk, 1953; Vishniac & Santer, 1957; Santer, Boyer & Santer,

* Present address: Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T., Australia.

1959; Jones & Happold, 1961). From time to time, however, there have been reports that some strains of the aerobic bacterium, *Thiobacillus thioparus*, are unable to metabolize this polythionate (e.g. Tamiya, Haga & Huzisige, 1941; Parker & Prisk, 1953).

During a study of tetrathionate oxidation by *T. thioparus* and a closely related bacterium, *Thiobacillus X*, it was observed that the results were markedly influenced by a number of experimental conditions. The effects of some of these conditions, notably oxygen concentration and the presence of thiosulphate, are described in the present paper.

MATERIALS AND METHODS

Organisms. The bacteria used in this study were *Thiobacillus X* (Parker & Prisk, 1953) and a strain of *T. thioparus*, a gift from Dr M. Santer and originally obtained from the collection of Dr R. L. Starkey. The methods of maintenance and culture have been described by Trudinger (1961a). For experiments, the cells were centrifuged, washed twice with 0.1 M-potassium phosphate, pH 7, and suspended in the same buffer.

Tetrathionate. Unlabelled potassium tetrathionate, $K_2S_4O_8$, was prepared by the oxidation of thiosulphate with iodine as described by Trudinger (1961b). Considerable losses were experienced when the recrystallization of the potassium salt on a milligram scale was attempted. The following modified procedure was used, therefore, for the preparation of $[^{35}S_2]$ tetrathionate. A 1:1 mixture of inner and outer labelled $[^{35}S]$ thiosulphate with equal specific activities, obtained from The Radiochemical Centre, Amersham, Bucks., was oxidized with a slight excess of iodine, and the $[^{35}S_2]$ -tetrathionate was precipitated by the addition of barium acetate and an equal volume of ethanol. The barium $[^{35}S_2]$ -tetrathionate was then recrystallized twice from water-ethanol mixtures. The average yield was about 85%. Solutions of potassium $[^{35}S_2]$ tetrathionate were prepared by double decomposition of the barium salt with K_2SO_4 . These showed a single radioactive spot when analysed by paper electrophoresis.

Paper electrophoresis of radioactive inorganic sulphur compounds. The method described by Trudinger (1961b) was used. Radioactive areas were located by exposing the paper strips to Kodirex X-ray film. For quantitative determination of radioactivity, measured amounts (usually 5–10 μ l.) of material were separated by paper electrophoresis, and the radioactive areas were cut out and digested overnight at room temperature with a mixture of 25 ml. of conc. HNO_3 saturated with bromine and 5 ml. of conc. HCl . The following day the mixture was evaporated to dryness on a sand bath over a period of about 5 hr. This procedure converted all sulphur into sulphate and the organic material into a soluble form. The dry material was dissolved in water and samples were plated for the assay of ^{35}S (Trudinger, 1961b).

Manometry. The conventional Warburg manometric technique was used. Flasks of approx. 15 ml. volume were shaken at 136 oscillations/min. and an amplitude of 2.5 cm. Unless otherwise stated, experimental mixtures contained 200 μ moles of potassium phosphate, pH 7, in a volume of 1.8 ml. and were incubated at 30° in air: 0.2 ml. of 2N-NaOH and fluted filter paper were placed in the centre well.

Limiting oxidation rates. These are the highest rates of oxidation found under the conditions used in the Warburg experiments and presumably correspond to the rates at which oxygen diffusion becomes limiting (cf. Umbreit, Burris & Stauffer, 1957).

RESULTS

Effect of cell concentration and storage on tetrathionate oxidation. Maximum rates of tetrathionate oxidation in air were obtained only after a lag period, the extent of which increased as the cell concentration was lowered, and when the cells were aged at 2° after harvesting (Fig. 1). Moreover, until oxygen diffusion became limiting, Q_{O_2} values increased markedly as the cell concentration was raised, the highest values being obtained when the rate of oxygen uptake was above 65–70% of the limiting oxidation rate for these experimental conditions (Table 1). Below a certain cell concentration, which varied with the particular cell preparation and the strain of organism, tetrathionate oxidation ceased altogether or proceeded at a very low rate.

Effect of thiosulphate on tetrathionate oxidation. Previous studies have indicated that tetrathionate may be an intermediate in the oxidation of thiosulphate to sulphate by thiobacilli (Vishniac, 1952; Vishniac & Santer, 1957; Trudinger, 1959; Santer, Margulies, Klinman & Kaback, 1960; Jones & Happold, 1961). In the present work, however, low concentrations of cells, which failed to oxidize tetrathionate or did so only after extensive lag periods, rapidly oxidized thiosulphate without a lag phase, and the amounts of oxygen taken up were much in excess of that required for the oxid-

Fig. 1. Effect of cell concentration and storage on tetrathionate oxidation. The reaction mixtures contained *T. thioparus* and 10 μ moles of $K_2S_4O_8$. Cell concentrations (mg. dry wt.) were: curves 1 and 3, 4.2; curves 2 and 4, 2.1; curve 5, 1.05; curve 6, 0.5. Curve 7 was obtained with 4.2 mg. of cells in the absence of $K_2S_4O_8$. Solid lines, fresh cells; broken lines, cells aged 24 hr. in 0.1 M-phosphate. pH 7.

ation of thiosulphate to tetrathionate. The effect of thiosulphate on tetrathionate oxidation was therefore examined.

At relatively high cell concentrations in air thiosulphate 'sparked' tetrathionate oxidation which then proceeded at a rapid rate until all the substrate was oxidized (Fig. 2). Thiosulphate was active when added to the reaction medium together with tetrathionate (Fig. 2, curve 1) or at some later stage (Fig. 2, curve 3a), but, when tetrathionate was added after the oxidation of thio-

Table 1. *Effect of cell concentration on the rate of tetrathionate oxidation*

The reaction mixtures contained 0.2 μ mole of $\text{Na}_2\text{S}_2\text{O}_3$ [added to 'spark' tetrathionate oxidation (see the text)] and 20 μ moles of $\text{K}_2\text{S}_4\text{O}_6$. Expt. 1: *Thiobacillus X*; volume 2.3 ml. Expt. 2: *T. thioparus*; volume 1.8 ml. The limiting oxidation rates were approx. 900 for Expt. 1 and 1200 for Expt. 2.

Expt. no.	Cells (mg. dry wt.)	Rate of O_2 uptake ($\mu\text{l./hr.}$)	Max. Q_{O_2}
1	0.9	0	0
	1.8	90	50
	4.5	690	154
	9.0	893	99
	0.5	20	40
	1.0	225	225
	2.1	855	406
	4.2	1200	286

Fig. 2. 'Sparking' of tetrathionate oxidation by thiosulphate. *Thiobacillus X* (6.0 mg. dry wt.) was incubated with 20 μ moles of $\text{K}_2\text{S}_4\text{O}_6$ or 1 μ mole of $\text{Na}_2\text{S}_2\text{O}_3$ or both. Curves: 1, $\text{K}_2\text{S}_4\text{O}_6 + \text{Na}_2\text{S}_2\text{O}_3$, tipped at 10 min.; 2, $\text{Na}_2\text{S}_2\text{O}_3$ at zero time and $\text{K}_2\text{S}_4\text{O}_6$ at 10 min.; 3, $\text{K}_2\text{S}_4\text{O}_6$ at 10 min.; 3a, $\text{K}_2\text{S}_4\text{O}_6$ at 10 min. and $\text{Na}_2\text{S}_2\text{O}_3$ at the time indicated by the arrow; 4, no $\text{K}_2\text{S}_4\text{O}_6$ or $\text{Na}_2\text{S}_2\text{O}_3$ added. Curves 1 and 3a proceeded at an approximately linear rate until all substrate was oxidized.

sulphate was complete, no 'sparking' of tetrathionate oxidation was observed (Fig. 2, curve 2). At lower cell concentrations rapid oxidation of thiosulphate-tetrathionate mixtures ceased soon after the oxidation of thiosulphate alone stopped (Fig. 3, curves 3, 4 and 7). The total oxygen consumed in the presence of the mixture of thiosulphate and tetrathionate, however, was consistently greater than the sum of the oxygen uptakes when thiosulphate and tetrathionate were metabolized separately for the same period of time. There was no constant relationship between the amounts of thiosulphate added and the excess of oxygen consumption: in a series of six experiments the excess of oxygen consumption varied from 30 to 110% when 1 μ mole of thiosulphate and 10 μ moles of tetrathionate were oxidized. Analyses of the products of oxidation of mixtures of thiosulphate and $^{35}\text{S}_2$ tetrathionate showed that, when rapid oxygen uptake stopped, only sulphate and tetrathionate remained in the medium, and that small amounts of tetrathionate had been metabolized.

With cell concentrations sufficiently high to allow complete oxidation of tetrathionate, this

Fig. 3. Effect of cell concentration and oxygen on the 'sparking' action of thiosulphate. *Thiobacillus X* was incubated with 10 μ moles of $\text{K}_2\text{S}_4\text{O}_6$ or 1 μ mole of $\text{Na}_2\text{S}_2\text{O}_3$ or both with either air (solid lines) or 100% O_2 (broken lines) as the gas phase. Curves: 1 and 2, $\text{K}_2\text{S}_4\text{O}_6 + \text{Na}_2\text{S}_2\text{O}_3$, 2.9 mg. of cells; 3, $\text{K}_2\text{S}_4\text{O}_6 + \text{Na}_2\text{S}_2\text{O}_3$, 1.2 mg. of cells; 4 and 5, $\text{Na}_2\text{S}_2\text{O}_3$, 1.2 mg. of cells; 6, $\text{K}_2\text{S}_4\text{O}_6$, 2.9 mg. of cells; 7, $\text{K}_2\text{S}_4\text{O}_6$, 1.2 mg. of cells. The curves for the oxidation of $\text{K}_2\text{S}_4\text{O}_6$ in 100% O_2 have not been included in the Figure but were similar to curves 6 and 7 for 2.9 and 1.2 mg. of cells respectively. No O_2 consumption occurred in the absence of substrate.

oxidation was 'sparked' by thiosulphate at a concentration of about $0.1 \mu\text{mole/ml}$. Above this concentration the rate of the 'sparked' oxidation of tetrathionate was independent of the amount of thiosulphate added: in no instance has a partial activation of tetrathionate oxidation been observed.

Specificity of the 'sparking' effect. Of the compounds tested, thiosulphate was the most effective 'sparker' of tetrathionate oxidation. Sulphide, sulphite, pentathionate, cysteine and reduced glutathione were active at concentrations of $1\text{--}2 \mu\text{moles/ml}$. Qualitative analyses by paper electrophoresis showed, however, that, in all cases, significant amounts of thiosulphate were rapidly produced either biologically (from pentathionate) or by chemical reaction with tetrathionate (from sulphide, sulphite, cysteine and glutathione), and it was not possible to determine whether these materials themselves had any 'sparking' action. Trithionate, cystine, oxidized glutathione, ascorbate and colloidal sulphur were not active at concentrations of up to $5 \mu\text{moles/ml}$. Colloidal sulphur was itself oxidized under these conditions.

Effect of oxygen concentration on tetrathionate oxidation. As mentioned above the highest values for Q_{O_2} for tetrathionate oxidation were found when the rate of oxygen uptake was greater than about 65–70 % of the limiting oxidation rate (e.g. Table 1): under these conditions the concentration of dissolved oxygen fell to less than $2 \mu\text{M}$ as measured with the Clark oxygen electrode (Yellow Springs Instrument Co., Ohio). The possibility that a low oxygen concentration in the medium is necessary for rapid tetrathionate oxidation was tested by studying the effects of variations of oxygen concentration in the gas phase.

Tetrathionate oxidation was strongly inhibited by a gas phase of 100 % oxygen, the rate of oxidation in this gas phase being about the same as that during the lag period in air. In Fig. 3 (curves 1 and 2) the effect of thiosulphate on tetrathionate oxidation in 100 % oxygen is shown. The results were similar to those obtained when mixtures of tetrathionate and thiosulphate were oxidized by lower cell concentrations in air; the rate of oxidation decreased markedly soon after thiosulphate oxidation was complete and the total oxygen uptake by the mixture at this point exceeded the sum of the oxygen consumption for thiosulphate and tetrathionate metabolized separately. Fig. 4 shows the effect of a decrease in oxygen concentration on the oxidation of tetrathionate by freshly prepared *Thiobacillus X*. At the three highest cell concentrations in air the addition of the 'sparking' amount of thiosulphate was necessary for maximum rates of tetrathionate oxidation. In 5 % oxygen the rates of tetrathionate oxidation in the absence of thiosulphate were several times greater

than that in air and there was little or no increase in the rates of oxidation on the addition of thiosulphate. At the lowest cell concentration (Fig. 4, curves 4, 8, 4a and 8a) there was no significant oxidation of tetrathionate either in air or 5 % oxygen. Cells aged for 2 days at 2° required the addition of thiosulphate for the maximum oxidation of tetrathionate in both air and 5 % oxygen. Nevertheless, in 5 % oxygen 'sparking' occurred at lower cell concentrations than in air (Fig. 5, curves 4 and 3). A further decrease of the oxygen concentration to 1 % increased the rate of tetrathionate oxidation, this rate being unaffected by the addition of thiosulphate (Fig. 5, curves 5 and 6).

Anaerobic production of thiosulphate from tetrathionate. Under anaerobic conditions tetrathionate was metabolized by *Thiobacillus X* with the production of thiosulphate (Table 2) which was identified by paper electrophoresis. Sulphate, elemental sulphur and smaller amounts of pentathionate and trithionate were also formed. The rate of this reaction was comparable with that of the maximum rate of tetrathionate oxidation, in the presence of a 'sparking' agent, and was not affected by the cell concentration.

Fig. 4. Effect of decreased oxygen concentrations on tetrathionate oxidation by fresh *Thiobacillus X*. Freshly prepared *Thiobacillus X* was incubated with $20 \mu\text{moles}$ of $\text{K}_2\text{S}_4\text{O}_6$ (solid lines) or with $\text{K}_2\text{S}_4\text{O}_6 + 0.5 \mu\text{mole}$ of $\text{Na}_2\text{S}_2\text{O}_3$ (broken lines). The gas phase was either air (i) or 5 % O_2 in N_2 (ii). Cell concentrations: curves 1, 5, 1a and 5a, 10 mg. ; curves 2, 6, 2a and 6a, 5 mg. ; curves 3, 7, 3a, and 7a, 2 mg. ; curves 4, 8, 4a and 8a, 0.4 mg.

Products of tetrathionate oxidation. The compounds produced during the early stages of oxidation of $[^{35}\text{S}_2]\text{tetrathionate}$ by a high cell concentration of *Thiobacillus X* are shown in Fig. 6. Tetrathionate was rapidly transformed into sulphate with only minor accumulations of thiosulphate, trithionate and pentathionate. In the experiment described, significant amounts of elemental sulphur were precipitated. The production of elemental sulphur was, however, variable and in many experiments was below the level of detection.

Comparison between Thiobacillus X and T. thioparus. The results reported above were qualitatively the same for both *Thiobacillus X* and *T. thioparus*, but there were some quantitative differences between the two organisms. The maximum values for Q_{O_2} in air with tetrathionate as substrate rarely exceeded 200 for *Thiobacillus X*, but values of 400–500 were common for *T. thioparus*. On the other hand, *Thiobacillus X* was less sensitive to storage than *T. thioparus*. Under con-

ditions allowing high rates of tetrathionate oxidation in the presence of thiosulphate *T. thioparus* invariably achieved this rate, after a lag period, in the absence of thiosulphate, but in many experiments with *Thiobacillus X* low rates of oxidation

Table 2. Anaerobic formation of thiosulphate from tetrathionate

Thiobacillus X (40 mg. dry wt.) was incubated at 30° under O_2 -free N_2 , with 100 μmoles of $\text{K}_2\text{S}_4\text{O}_6$ and 1 m-mole of potassium phosphate, pH 7, in a volume of 11 ml. At intervals 1 ml. samples were pipetted into 1 ml. of 10% (w/v) acetic acid, and thiosulphate was determined by titration with standard iodine with starch as an indicator. The results have been corrected for a small blank found with cells alone. No thiosulphate was produced by cells heated at 60° for 5 min.

Time (min.)	Thiosulphate formed (μmoles)
2	9.2
4	18.4
6	26.4
10	36.0
15	47.1
20	58.0

Fig. 5. Effect of decreased oxygen concentrations on tetrathionate oxidation by aged cells. *Thiobacillus X*, aged 48 hr. at 2° in 0.1M-phosphate, pH 7, was incubated with 20 μmoles of $\text{K}_2\text{S}_4\text{O}_6$. The gas phases and cell concentrations were as follows: curve 1, 20% O_2 , 6 mg. of cells; curve 2, 5% O_2 , 6 mg. of cells; curve 3, 5% O_2 , 3 mg. of cells; curve 4, 20% O_2 , 3 mg. of cells; curve 5, 1% O_2 , 6 mg. of cells; curve 6, 1% O_2 , 3 mg. of cells. For curves 1–4 0.5 μmole of $\text{Na}_2\text{S}_2\text{O}_3$ was added at the time indicated by the arrow. Curves 5 and 6 show O_2 uptakes in 1% O_2 with (Δ) or without ($\circ$) thiosulphate. The rates of O_2 uptake in curves 5 and 6 fell off rapidly after 30 min., presumably because over 50% of the total available O_2 had been used by this time.

Fig. 6. Products of tetrathionate oxidation. The experimental mixture contained 20 μmoles of $[^{35}\text{S}_2]\text{K}_2\text{S}_4\text{O}_6$, 15 mg. of *Thiobacillus X* and 200 μmoles of potassium phosphate, pH 7, in a volume of 3 ml. in a 15 ml. conical flask. The flask was shaken in air at 30°, and 0.3 ml. samples were taken at 2 min. intervals and pipetted into 0.2 ml. of ethanol to stop the reaction. The cells and any elemental sulphur were removed by centrifuging and samples of the supernatants analysed by paper electrophoresis as described in the Materials and Methods section. The precipitates were washed three times with 0.1M-phosphate and digested with HNO_3 – HCl – Br for the determination of elemental sulphur. Curves: 1, tetrathionate; 2, sulphate; 3, elemental sulphur; 4, thiosulphate; 5, trithionate + pentathionate.

were maintained for several hours in the absence of thiosulphate, although, on the addition of the latter, the rate of oxidation changed immediately to a high value.

DISCUSSION

The variations in tetrathionate oxidation with experimental conditions described in the present paper may help to explain the reports, mentioned above, that *T. thioparus* and related organisms are unable to oxidize this polythionate.

The effects of cell concentration on tetrathionate oxidation and its inhibition by 100 % oxygen and stimulation by low oxygen concentrations (Figs. 4 and 5) are consistent with the hypothesis that a low concentration of dissolved oxygen is necessary for maximum rates of oxidation.

The 'sparking' effect of thiosulphate on tetrathionate oxidation is apparently related to this phenomenon since it is abolished by 100 % oxygen (Fig. 3) and, under certain conditions, is duplicated by the lowering of the oxygen concentration (Figs. 4 and 5).

Thiosulphate was active as a 'sparking' agent at a concentration of $0.1 \mu\text{mole/ml.}$, the complete oxidation of which would consume $4.5 \mu\text{l.}$ of oxygen/ml. Air-saturated buffer contains approx. $5 \mu\text{l.}$ of oxygen/ml. at 30° : complete and almost instantaneous oxidation of the thiosulphate, therefore, would have been necessary to account for the 'sparking' effect in terms of a momentary lowering of oxygen concentration in the medium. Moreover, tetrathionate oxidation was not 'sparked' by elemental sulphur which itself was oxidized by the cells, and, throughout many experiments, no correlation has been found between the presence or absence of an endogenous oxidation and the ability of cells to oxidize tetrathionate in the absence of a 'sparking' agent. A more specific role for thiosulphate is therefore indicated; the other compounds found to reproduce the effect of thiosulphate do so only at much higher concentrations and only under conditions that lead to the formation of significant amounts of thiosulphate either by chemical reactions with tetrathionate or by metabolic reactions.

The formation of small amounts of thiosulphate during the oxidation of tetrathionate by high cell concentrations (Fig. 6) may account for the continuation of tetrathionate oxidation once initiated. Thiosulphate is produced from tetrathionate anaerobically (Table 2) and, since any thiosulphate formed would be immediately oxidized, it is clear that this reaction must be inhibited in air. Should the anaerobic reaction be concerned also with tetrathionate oxidation this may account for the apparent requirement for a low concentration of dissolved oxygen for rapid tetrathionate oxidation.

An explanation for the mode of action of thiosulphate must await more definite evidence for the initial steps in tetrathionate oxidation over which there is much controversy. Tamiya *et al.* (1941), Vishniac (1952) and Jones & Happold (1961) have proposed that tetrathionate is first oxidized to trithionate, and an initial reduction of tetrathionate to thiosulphate has been suggested by Ostrowski & Krawczyk (1957) although no evidence was presented in support of this hypothesis. The latter explanation has been used by Peck & Fisher (1962) to account for the oxidation of tetrathionate by extracts of *T. thioparus*: in this case, however, the reduction probably occurred by a direct chemical reaction between tetrathionate and reduced glutathione which was added to their system. A variation of the reduction theory has been put forward by Lees (1960) and Vishniac & Trudinger (1962), who have speculated that tetrathionate may be split to thiosulphate and an organic sulphenyl thiosulphate by a reaction with a thiol group in the cell. The evidence available at the present time does not point conclusively to any one mechanism of tetrathionate oxidation.

At low cell concentrations, or high oxygen concentrations, thiosulphate was rapidly oxidized well beyond the stage of tetrathionate, although the rate of oxidation of the latter was very low or negligible. This, at first sight, indicates that tetrathionate cannot be an intermediate in thiosulphate oxidation (cf. Ostrowski & Krawczyk, 1957; Peck, 1960). The experiments described in Fig. 3, however, show that some tetrathionate was metabolized concurrently with thiosulphate oxidation, when tetrathionate and thiosulphate were incubated with *Thiobacillus X* under non-'sparking' conditions. Thus, in the oxidation of thiosulphate alone under these conditions, any tetrathionate formed would be expected to be metabolized further until all thiosulphate disappeared from the medium. The possibility that tetrathionate is an intermediate in thiosulphate oxidation, therefore, is not eliminated by these results.

SUMMARY

1. Tetrathionate was oxidized by *Thiobacillus X* and *T. thioparus* only after a lag period the extent of which was determined by the age, strain and concentration of the cells.

2. At high cell concentrations tetrathionate oxidation was 'sparked' by the addition of a trace amount of thiosulphate.

3. At low cell concentrations thiosulphate did not 'spark' tetrathionate oxidation but some tetrathionate was metabolized concurrently with thiosulphate oxidation.

4. The 'sparking' action of thiosulphate was inhibited by 100 % oxygen. Decreased oxygen con-

centrations allowed tetrathionate oxidation to proceed without the addition of thiosulphate.

5. Tetrathionate was metabolized anaerobically with the formation of thiosulphate, and small amounts of the latter were present in reaction mixtures that were actively oxidizing tetrathionate.

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Uronic Acid Constituents of Oat-Coleoptile Cell Walls

By P. M. RAY AND D. A. ROTTENBERG

Department of Botany, University of Michigan, Ann Arbor, Mich., U.S.A.

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The role of cell-wall polysaccharides containing uronic acid, and specifically of pectin, in the growth of plant cells has been emphasized in investigations on growing oat-coleoptile tissue (Ordin, Cleland & Bonner, 1955, 1957; Albersheim & Bonner, 1959; Jansen, Jang, Albersheim & Bonner, 1960). It seems to be widely assumed that the uronic acid content of primary cell-wall materials is due simply to their content of pectin; for example, Jansen *et al.* (1960) reported detecting only galacturonic acid by a pectinase extraction of oat-coleoptile cell-wall material that was said to have brought all uronic acid into solution. However, Bishop, Bayley & Setterfield (1958) obtained evidence indicating the presence of glucuronic acid as well as galacturonic acid.

In the present work we have investigated the uronic acid composition and content of oat-coleoptile cell-wall material in some detail and have found it to be considerably more complex than has hitherto been recognized.

RESULTS

Isolation and identification of uronic acid components. Uronic acids were isolated from acid hydrolysates of polysaccharides of oat-coleoptile

cell walls by adsorption on Dowex 1, followed by chromatography in two solvents, by means of which not only could free glucuronic acid and galacturonic acid be separated from one another but a number of other components could be isolated. The acids are listed in Table 1, and the yields obtained in various experiments are shown in Table 2.

In most cases whole coleoptile cell walls were extracted with hot 0.05N-sulphuric acid on the expectation that, since hot dilute mineral acid has been said to extract the 'protopectin' of this cell-wall material (Ordin *et al.* 1955, 1957), the bulk of the uronic acid should be obtained by this solvent. In one case we extracted and examined separately the fraction soluble in hot water ('pectin'). In another experiment we hydrolysed hemicellulose B, prepared from oat-coleoptile cell-wall material in the usual manner.

Most of the products appeared to be hydrolysis-resistant aldobiouronic acids and other acidic oligosaccharides and were identified as follows: each was subjected to further hydrolysis with sulphuric acid, followed by separation of acidic from neutral products with Dowex 1. Sugar and uronic acid products were identified by chromatography (Table 1), by a procedure that distinguished