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The Reduction of Sulphur Compounds by *Desulphovibrio desulphuricans*

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SUMMARY: *Desulphovibrio desulphuricans*, strain 'Hildenborough', was able to use sulphite, thiosulphate, tetrathionate, metabisulphite or dithionite in place of sulphate for growth. Resting cell suspensions reduced these ions using the theoretical amounts of hydrogen and forming the theoretical amount of sulphide, except in the case of dithionite, which probably decomposed spontaneously to sulphate and sulphur before being reduced.

The organism was unable to grow with or to reduce dithionate, perdisulphate, 'formaldehydesulphoxylate', sulphamate, benzenesulphonate, methanesulphonate, β -hydroxyethane-sulphonate, sodium ethylsulphate, dimethylsulphone or cystine. Elementary sulphur, if purified by re-distillation, was also not attacked. Five other strains of *D. desulphuricans*, four of them cultivated autotrophically, were also unable to grow with pure elementary sulphur.

Colloidal sulphur permitted slow growth, or slow hydrogen absorption when a resting cell suspension was used. This effect was not due to oxide impurities in the sulphur permitting growth, as an ultra-filtrate of colloidal sulphur had considerably less activity.

A study of the rates of H_2 uptake suggested that sulphur, thiosulphate and tetrathionate were not intermediates in normal sulphate reduction, but that sulphite was.

The sulphate-reducing bacteria were first described by Beijerinck (1895), and were subsequently investigated by many workers including van Delden (1903), Baars (1930), Starkey (1938), Butlin & Adams (1947), ZoBell & Rittenberg (1948) and Butlin, Adams & Thomas (1949*b*). 'Growth' was usually estimated by the amount of black ferrous sulphide precipitate formed by reaction of the sulphide produced from the reduction of sulphate with the excess of ferrous salt added to the medium. Butlin, Adams & Thomas (1949*b*) showed that this large amount of iron is unnecessary, though traces insufficient to form a visible precipitate of iron sulphide are needed for growth.

The media used for the cultivation of these organisms were based on the use of a carbon source such as lactic acid, glucose or, for growth in autotrophic conditions, bicarbonate. Except in the latter case the carbon source acted also as a hydrogen donor for sulphate reduction, and the ability of various organic compounds to act as hydrogen donors was studied by Baars (1930). Butlin & Adams (1947) showed that, in autotrophic media, mild steel could act as a hydrogen donor, due to the formation of an electrolytic film of hydrogen at its surface in contact with the medium. Stephenson & Stickland (1931) showed that a strain of this organism was able to utilize gaseous hydrogen, and that sulphate reduction proceeded quantitatively according to the equation:

Postgate (1949) reported similar results with washed cell suspensions which reduced sulphate, sulphite and thiosulphate. Baars (1930) found that sulphate-reducing bacteria would grow with sulphite, thiosulphate, 'hydrosulphite' (dithionite) or colloidal sulphur in place of sulphate, but not with 're-precipitated' sulphur. When studying marine halophilic strains ZoBell & Rittenberg (1948) added tetrathionate to this list.

Butlin, Adams & Thomas (1949*b*) showed that growth and sulphide formation occurred with sulphate, sulphite, thiosulphate, and, in contrast to Baars (1930), also with 're-precipitated' sulphur, in autotrophic as well as heterotrophic conditions.

Evaluation of growth by sulphide formation may be unsatisfactory, since sulphide formation can take place without cell division, and this technique has probably been used only because of its ease and convenience. In media based on lactic acid and salts, growth without added iron is not abundant, but it is improved by the addition of yeast extract (Butlin, Adams & Thomas, 1949*b*). Recently, a medium giving vigorous growth without precipitation of iron sulphide has been described (Postgate, 1951) and used to grow large amounts of cells for manometric studies (Postgate, 1949). Using a sulphate-free modification of this medium, one can observe the growth of the organisms directly and investigate the ability of various compounds to replace sulphate for growth. Some studies of this kind are reported below, together with a quantitative investigation of the reduction of certain sulphur compounds by resting cell suspensions.

METHODS

Organism. A strain of *D. desulphuricans* called 'Hildenborough' was used in this work except where otherwise stated. Its origin and the routine methods of maintenance were described by Postgate (1951). Washed inocula were used for all growth tests recorded here.

Media and Materials. To study the ability of substances to replace sulphate it was desirable to have a medium which permitted optimal growth in the presence of sulphate and none in its absence. Such a medium was not easily prepared because most peptone preparations contain sulphate. A medium was found, however, based on Difco 'Bacto-Tryptone' (Baird & Tatlock Ltd., London), which permitted only very limited growth in the absence of added sulphate, owing probably to its low content of inorganic sulphur ('Difco Manual', 1948). This medium contained 'Bacto-Tryptone', 5 g.; glucose, 10 g.; yeast extract (Difco), 4 g.; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.8 g., in 1 litre of distilled water; pH 7.2. This will be referred to as the *complex medium*.

For certain tests it was necessary that no growth at all should occur in the absence of added substrate, and for this purpose a modification of Starkey's medium (Starkey, 1938) was used, having the composition: KH_2PO_4 , 0.5 g.; NH_4Cl , 1 g.; $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g.; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.1 g.; sodium lactate (British Drug Houses Ltd., 70% w/w) 5.0 g., in 1 litre of distilled water; pH 7.5. This medium gave only limited growth in the presence of sulphate owing

to the absence of complex organic supplements. It will be referred to as the *simple medium*.

Where possible, materials of 'Analar' grade were used, but in certain cases (mentioned in the text) such specimens were not available. Sodium tetrathionate was at first prepared from an ethanolic solution of iodine and sodium thiosulphate, using the method described by Partington (1946), and was reprecipitated twice with absolute ethanol before use. This procedure did not give a pure preparation, as is mentioned later. 'Analar' grade $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$ was recrystallized.

Substances to be tested were usually sterilized by autoclaving for 10 min. at 10 lb./sq. in., but when the substances might be unstable to heat they were added without sterilization. Such cases are mentioned; at the end of the test the cultures were always examined for contaminants, but none were found.

Resting Cell Suspensions. Cell suspensions for use in the Warburg manometer were prepared by centrifuging a culture and resuspending the cells in aqueous NaCl (0.8 % w/v) so as to give a suspension containing between 2 and 3 mg. dry wt. of cells/ml. Traces of substrate carried through the washing procedure were eliminated by pre-incubating the suspension for about 20 min. in a stream of hydrogen before use. One ml. of this suspension was added to the manometer vessel with 1 ml. of KH_2PO_4 buffer (0.5 % w/v, pH 6.3) and the substrate (usually 2.5 $\mu\text{mol.}$) was added in 0.5 ml. of water to the side arm; 0.5 ml. of aqueous NaOH (15 % w/v) or cadmium chloride (10 % w/v) were contained in the centre cup together with filter paper or glass wool. The manometer vessels were incubated at 37°. Six determinations of the $-\text{Q}_{\text{H}_2}$ for sulphate with a certain suspension gave a mean value of 323 $\text{mm}^3 \text{H}_2/\text{mg. dry cells/hr.}$ with a standard deviation of 29.25 $\text{mm}^3/\text{mg./hr.}$; five determinations of the hydrogen absorbed with *c.* 2.5 $\mu\text{mol.}$ of Na_2SO_4 gave a mean absorption of 276.6 $\text{mm}^3 \text{H}_2$ with a standard deviation of 2.72 $\text{mm}^3 \text{H}_2$.

Sulphide Estimations. Attempts to estimate the sulphide formed in the manometer vessel by analysis of the NaOH-soaked paper in the centre cup were unsuccessful, but a method using cadmium chloride in place of NaOH, and glass wool in place of filter paper proved satisfactory and was used throughout this work.

The manometer vessels were set up with a pre-incubated cell suspension and phosphate buffer in the main compartment and substrate in the side-arm. A small plug of acid-cleaned glass wool was placed in the centre cup and 0.5 ml. of aqueous CdCl_2 (10 % w/v) was added. At the end of the experiment the plug was transferred with clean tweezers to a stoppered titration vessel containing 1 ml. of 0.1 N iodine and 4 ml. of 5 N-HCl, which was shaken and left for 5 min. The centre cup was then rinsed with the titration liquid, followed by distilled water, to remove the last traces of sulphide, and the washings were added to the titration vessel. The whole volume was back-titrated against 0.01 N sodium thiosulphate, using a drop of carbon tetrachloride as an internal indicator. This method gave satisfactory results down to about 0.3 $\mu\text{mol.}$ of sulphide, provided the titration fluid was left until all

CdS had decomposed (5 min.), and provided the titration vessel was kept stoppered as much as possible to prevent back-oxidation of HI. The standard deviation calculated from 12 readings taken over some months was $0.1273 \mu\text{mol}$. of sulphide. The use of CdCl_2 in place of NaOH in the manometer made no difference to the amount of gas absorbed with a given substrate, indicating that no CO_2 is formed during sulphate reduction and that no correction need be applied for this.

RESULTS

Growth Experiments

Without substrate. No growth occurred in sulphate-deficient simple medium, but some growth occurred in the sulphate-deficient complex medium. This 'blank' growth was probably due to sulphate and other reducible compounds present in the 'Bacto-Tryptone' and yeast extract; indeed, the former is known to contain 0.04 % of inorganic sulphur ('Difco Manual', 1948).

Sulphate. Sulphate is the natural hydrogen-acceptor for the growth of *D. desulphuricans*. In the sulphate-deficient complex medium the growth of the organism depended linearly on the sulphate concentration between about 0.5 and $10 \mu\text{mol}$. sulphate/ml. Higher concentrations of sulphate depressed the growth rate to some extent, though the maximum stationary population was ultimately reached (Fig. 1). Optimal growth was given by $10 \mu\text{mol}$ s sulphate/ml., and this concentration was therefore used for testing other compounds, except in the case of elementary sulphur (see below).

Fig. 1. Effect of sodium sulphate concentration on the growth of *D. desulphuricans* (Hildenborough) in a medium of glucose tryptone and yeast extract. ○ growth after 142 hr. incubation; ● growth after 92 hr. incubation.

Sulphite. Sodium sulphite could replace sulphate for the growth of *D. desulphuricans* in the sulphate-deficient complex medium (Table 1). It has been used in other media, at a concentration of 3 %, to purify crude cultures (Butlin, Adams & Thomas, 1949*b*) and has been observed to cause considerable elongation of the cells (Butlin, Adams & Thomas, 1949*a*).

Thiosulphate. Sodium thiosulphate could replace sodium sulphate for growth of the organism in the sulphate-deficient complex medium (Table 1).

Tetrathionate. Sodium tetrathionate could replace sodium sulphate for growth of the organism in sulphate-deficient complex medium, but growth took place more slowly (Table 1).

Table 1. *Growth of D. desulphuricans (Hildenborough) on a medium of glucose, peptone and yeast extract containing various substances in place of sulphate*

Test substance	Concentration M	Growth after (hr.)			
		24	48	72	96
Sulphate Na_2SO_4	10^{-2}	tr.	+	+++++	+++++
Sulphite Na_2SO_3	10^{-2}	tr.	+	+++++	+++++
Thiosulphate $\text{Na}_2\text{S}_2\text{O}_3$	10^{-2}	tr.	+	+++	+++++
Dithionate $\text{Na}_2\text{S}_2\text{O}_6$	10^{-2}	—	tr.	tr.	tr.
*Tetrathionate $\text{Na}_2\text{S}_4\text{O}_6$	10^{-2}	tr.	tr.	++	+++++
*Dithionite $\text{Na}_2\text{S}_2\text{O}_4$	10^{-2}	—	tr.	+++	+++++
Perdisulphate $(\text{NH}_4)_2\text{S}_2\text{O}_8$	10^{-2}	—	tr.	tr.	tr.
*Formaldehyde sulphonylate $\text{NaHSO}_3 \cdot \text{HCHO}$	10^{-2}	—	tr.	tr.	tr.
*Metabisulphite $\text{K}_2\text{S}_2\text{O}_5$	10^{-2}	tr.	tr.	++	+++
Benzenesulphonate $\text{NaSO}_3 \cdot \text{C}_6\text{H}_5$	10^{-2}	—	tr.	tr.	tr.
Sulphur (B.D.H., re-ppt.) S	$\frac{1}{4} \times 10^{-2}$	—	tr.	+	+
*Sulphamate $\text{NH}_4 \cdot \text{SO}_3\text{NH}_2$	10^{-2}	—	tr.	tr.	tr.
Methanesulphonate $\text{NaO} \cdot \text{SO}_3 \cdot \text{CH}_3$	10^{-2}	—	tr.	tr.	tr.
β -Hydroxyethanesulphonate $\text{NaO} \cdot \text{SO}_3 \cdot \text{CH}_2\text{CH}_2\text{OH}$	10^{-2}	—	tr.	tr.	tr.
†Sulphide Na_2S	10^{-2}	tr.	tr.	tr.	tr.
No addition	...	—	tr.	tr.	tr.
Sulphate Na_2SO_4	10^{-2}	—	tr.	+++++	+++++
†Ethylsulphate $\text{Na}(\text{C}_2\text{H}_5)\text{SO}_4$	10^{-2}	—	—	—	tr.
Dimethylsulphone $(\text{CH}_3)_2\text{SO}_2$	10^{-2}	—	—	—	tr.
No addition	...	—	—	—	tr.

* Added without sterilization.

† Seitz filtered.

‡ Added as suspension.

Dithionite. Sodium dithionite ('hydrosulphite' British Drug Houses Ltd.) replaced sulphate for the growth of the organism (Table 1), but it is likely that at the test pH value of 7.2, most of the $\text{S}_2\text{O}_4^{2-}$ ion had decomposed spontaneously. Solutions of the specimen used decomposed slowly in water with deposition of sulphur, and were therefore made up immediately before use and added without sterilization. Nevertheless, within 1 hr. a precipitate of sulphur was visible in the test medium, and it is likely that sodium dithionite supported growth because it decomposed spontaneously to sulphate.

Metabisulphite. It was thought desirable to test potassium metabisulphite since the $\text{S}_2\text{O}_5^{2-}$ is known to exist as a separate ionic species in solution, though whether it was stable in the test conditions was not known. It replaced sulphate for growth of the organism (Table 1), but growth took place slowly and was less extensive.

Sulphur. 'Re-precipitated' sulphur (British Drug Houses Ltd.) supported only limited growth of the organism, though a suspension of 40 μg . atoms

sulphur/ml. was used for testing in contrast to the 10 μ mol./ml. solutions used of other substances (Table 1). This low activity might have two causes: (a) the low solubility of the substrate or (b) inactivity of the sulphur *per se* masked by the presence of active impurities. To test the second possibility a specimen of sulphur was distilled in an all-glass apparatus with a stream of oxygen-free nitrogen, ground in a flamed mortar and tested at once, without further sterilization. It did not support the growth of *D. desulphuricans*, and, since the preparation was perfectly satisfactory for the aerobic growth of *Thiobacillus thio-oxidans*, this failure was not due to a change in the allotropic state of the sulphur such as to render it biologically inactive.

Since the inactivity of purified sulphur contrasted with the reports of previous workers, it was studied in more detail. Cultures were set up with excess iron (FeCl_2 , 0.5 mg./ml.), and also without, in sulphate-free simple medium and in sulphate-deficient complex medium. In the simple medium neither growth nor blackening occurred with the purified sulphur, and in the complex medium growth and blackening were equivalent to that obtained in the cultures without added substrate (Table 2). A test of the same kind using *D. desulphuricans* strain 'Teddington R' gave a similar result.

Table 2. *Growth and sulphide formation of D. desulphuricans (Hildenborough) with sulphate and re-distilled sulphur (1 mg./ml.)*

With sulphate-deficient complex medium	Effect after (hr.)				
	24	48	72	96	114
Sodium sulphate	—	++	++++	+++++	+++++
Re-distilled sulphur	—	—	tr.	tr.	tr.
—	—	—	tr.	tr.	tr.
Sodium sulphate + FeCl_2	*	*	*	*	*
Re-distilled sulphur + FeCl_2	—	—	*	*	*
FeCl_2	—	—	*	*	*
With sulphate-deficient lactate medium					
Sodium sulphate	—	+	++	+++	+++
Re-distilled sulphur	—	—	—	—	—
—	—	—	—	—	—
Sodium sulphate + FeCl_2	*	*	*	*	*
Re-distilled sulphur + FeCl_2	—	—	—	—	—
FeCl_2	—	—	—	—	—

* Sulphide formation detected by precipitation of FeS from FeCl_2 (0.5 mg./ml.) added to the culture medium.

Four strains cultured in autotrophic medium (Butlin, Adams & Thomas, 1949b) were also tested for their ability to utilize freshly-distilled sulphur, using the sulphate-deficient simple medium in which lactic acid was replaced by sodium bicarbonate. They were all tested in the presence of FeCl_2 (0.5 mg./ml.) so that growth was indicated by blackening of the culture. Since it was not convenient to wash the inocula, the inoculum cells for the test were grown with 're-precipitated' sulphur in place of sulphate. In no case was

growth or blackening detected in the presence of freshly-distilled sulphur. A specimen of re-distilled sulphur which had been left in air for about twenty days gave detectable blackening in autotrophic cultures.

It was possible that the sulphate-reducing bacteria were able to utilize sulphur only in the colloidal form—which was the form used by Baars (1930)—so a specimen of colloidal sulphur was obtained for testing in both simple and complex media. It supported the growth of both 'Hildenborough' and 'Teddington R' strains in these media. The specimen was known, however, to contain polythionic acid impurities equivalent to 0.0154 % $\text{H}_2\text{S}_5\text{O}_6$, and the activity of the preparation may have been due to these. An ultra-filtrate of the sulphur sol was obtained by passing it through a 30–55 m μ . A.P.D. collodion membrane under 2 atm. N_2 pressure, and this also was able to support growth of the bacteria, though it was optically free from sulphur. These findings are illustrated in Table 3. In some experiments the ultra-filtrate supported distinctly less growth than the sol, but quantitative comparisons were most unreliable since the growth was judged by blackening due to FeS precipitation. Direct comparisons of the growth obtained with the sulphur sol and its ultra-filtrate could not be made owing to the opacity of the former.

Table 3. *Sulphide formation by D. desulphuricans (Teddington R) with colloidal sulphur and its ultra-filtrate*

With sulphate-deficient complex medium	Effect after (hr).			
	24	48	72	96
Addition				
Sodium sulphate + FeCl_2	*	*	*	*
Colloidal sulphur + FeCl_2	—	*	*	*
Ultra-filtrate + FeCl_2	—	*	*	*
With sulphate-deficient lactate medium				
Sodium sulphate + FeCl_2	—	*	*	*
Colloidal sulphur + FeCl_2	tr.	*	*	*
Ultra-filtrate + FeCl_2	tr.	*	*	*

Other compounds. The following compounds were tested and did not support growth, nor did they prevent the 'blank' growth that occurred in the complex medium: sodium dithionate (British Drug Houses Ltd., recryst. $\times 2$), sodium formaldehyde sulphonylate (British Drug Houses Ltd., the only available example of a compound containing the ion SO_2^-), sodium benzenesulphonate (British Drug Houses Ltd.), ammonium perdisulphate, ammonium sulphamate (Baird & Tatlock), sodium methanesulphonate (synthetic), sodium β -hydroxyethanesulphonate (synthetic), sodium ethylsulphate (synthetic) and dimethylsulphone (synthetic). For completeness, sodium sulphide was tested as a substrate for growth and was inactive, though its presence accelerated the 'blank growth', owing possibly to its strong reducing properties. Growth tests with these substances are recorded in Table 1.

Hydrogen uptake with cell suspension

Without substrate. Resting cell suspensions took up hydrogen slowly over a long period in the absence of reducible substrates; the $-Q_{H_2}$ was c. $15 \text{ mm}^3/\text{mg.}/\text{hr.}$ (Fig. 3). In one experiment this 'blank' hydrogen uptake persisted for 48 hr., though the quotient was by then reduced.

Sulphide was not formed during the 'blank' hydrogen uptake, which, therefore, was unlikely to be due to the reduction of intracellular stores of sulphate-like material. Also, the rate of hydrogen absorption was unaffected by 10^{-3} M sodium selenate, which could have been sufficient to prevent the reduction of about $2 \times 10^{-2} \text{ M}$ sodium sulphate (Postgate, 1949).

Fig. 2. Effect of the age of cultures of *D. desulphuricans* (Hildenborough) on the specific rate of sulphate reduction.

Sulphate. Resting cell suspensions in the Warburg manometer absorbed 4 mol. hydrogen/mol. sulphate, and 1 mol. sulphide was formed (Table 2, Fig. 3). This is consistent with the work of Stephenson & Stickland (1931). The specific rate of hydrogen absorption ($-Q_{H_2}^{SO_4''}$) depended on the concentration of cells present, between 0.218 and 2.18 mg. dry wt. cells/ml., and was independent of the sulphate concentration between 10^{-3} and $10^{-1} \text{ M-Na}_2\text{SO}_4$. Charcoal, which might have provided a greater surface for inter-action between the gas and liquid phases, did not affect the quotient, nor did ferrous ions, which remove sulphide from solution as ferrous sulphide.

The effect of age of the cells on the specific rate of hydrogen absorption is indicated in Fig. 2. Cells taken from a culture less than 48 hr. after inoculation showed an enhanced $-Q_{H_2}^{SO_4''}$; this corresponded to the period of active cell-division in the growth medium. In the stationary phase, however, the quotient remained constant for several days, so cultures in this phase of growth were used for general comparative work. The $-Q_{H_2}^{SO_4''}$ of such cells was in the region of $200 \text{ mm}^3/\text{mg.}/\text{hr.}$ for *D. desulphuricans*, strain 'Hildenborough', though values between 150 and $350 \text{ mm}^3/\text{mg.}/\text{hr.}$ have been obtained with this strain.

The greater specific rate of hydrogen absorption during growth suggests

that sulphate reduction is the main energy-yielding reaction for cell multiplication; Subba Rao & Sreenivasaya (1947) have shown that the rate of sulphate utilization by a halophil is greater during active cell-division, which is consistent with this view.

Although several substances, including lactic acid and glucose can be used as hydrogen-donors for cell growth in the absence of hydrogen, they were not utilized by resting cell suspensions when gaseous hydrogen was available. Glucose (1 %, w/v), peptone (1 %, w/v) and yeast extract (1 %, w/v) had no effect on the rate of sulphate reduction or on the amount of hydrogen absorbed/ion of sulphate. Sodium lactate (0.35 %, w/v) decreased the $-Q_{H_2}^{SO_4}$ slightly but did not affect the total amount of hydrogen taken up.

Fig. 3. Hydrogen adsorbed by resting cells of *D. desulphuricans* (Hildenborough) with various substrates.

The curves shown were taken from several experiments and their slopes are not directly comparable. $-Q_{H_2}$ values for the curve are given below. 2.5 μ mol. of substrate were added in each test except in the case of sodium tetrathionate, when half this amount was used.

○ Sulphate, Na_2SO_4	210	● Dithionite, $Na_2S_2O_4$	220
● Sulphite, Na_2SO_3	210	● Thiosulphate, $Na_2S_2O_3$	164
● Metabisulphite, $K_2S_2O_5$	230	⊗ Tetrathionate, $Na_2S_4O_8$	60
● No added substrate	5.5		

Sulphite. Three mol. hydrogen were taken up/mol. Na_2SO_3 reduced (Fig. 3) and one mol. sulphide was formed (Table 2). For a given suspension, the $-Q_{H_2}^{SO_3}$ was greatest at pH 7.0, but it was only slightly lower at pH 6.3. The $-Q_{H_2}^{SO_3}$ was in general quantitatively similar to the $-Q_{H_2}^{SO_4}$ for the same strain, i.e. about 200 mm³/hr./mg., though on some occasions the sulphite quotient was found to be greater than the sulphate quotient.

Thiosulphate. Four mol. hydrogen were absorbed during the reduction of one mol. Na_2SO_3 (Fig. 3) and two mol. sulphide were formed (Table 4), corresponding to the equation:

The optimal pH value for thiosulphate reduction was 7.0 ± 0.2 , and the $-Q_{H_2}^{S_2O_4}$ of a given suspension under optimal conditions averaged about 60 % of the $-Q_{H_2}^{SO_4}$.

Table 4. *Sulphide formed and hydrogen absorbed by resting suspensions of D. desulphuricans (Hildenborough) with various substrates*

Substrate	Amount added ($\mu\text{mol.}$)	Hydrogen absorbed ($\mu\text{mol.}$)	Sulphide formed ($\mu\text{mol.}$)
Sulphate, Na_2SO_4	2.5	9.4 (10)	2.42 (2.5)
Sulphite, Na_2SO_3	2.5	7.3 (7.5)	2.40 (2.5)
Thiosulphate, $\text{Na}_2\text{S}_2\text{O}_3$	2.5	10.0 (10)	5.0 (5)
Tetrathionate, $\text{Na}_2\text{S}_4\text{O}_6$	1.25	11.2 (11.5)	4.82 (5)
Metabisulphite, $\text{K}_2\text{S}_2\text{O}_5$	1.5	8.9 (9)	3.0 (3)
Dithionite, $\text{Na}_2\text{S}_2\text{O}_4$	2.5	10.0 (10)	3.35 (2.5)

Theoretical figures are given in brackets.

Tetrathionate. The complete reduction of tetrathionate should theoretically take place in accordance with the equation:

but the uptake of 9 mol. of hydrogen proved difficult to demonstrate. Indeed, an early report from this laboratory (Report, 1950) stated that 7 mol. of hydrogen/tetrathionate ion were absorbed. This discrepancy was probably attributable to the difficulty experienced in preparing pure specimens of sodium tetrathionate, for a 97 % pure specimen which was obtained later gave hydrogen uptakes in accord with theory: 9 mol. of hydrogen were found to be absorbed/ion of tetrathionate added, and 4 mol. of sulphide were formed (Table 4). Even with this relatively pure specimen of sodium tetrathionate, however, the total hydrogen uptakes varied more than usual and hydrogen uptakes less than theoretical were not uncommon; for example, six parallel tests with the 97 % pure specimen gave hydrogen uptakes of 8.9, 8.45, 8.1, 9.4, 8.5 and 9.0 mol. of hydrogen/tetrathionate ion. The reason for these deviations is not known. The optimal pH for the reduction of tetrathionate was pH 8.2 and the optimal $-Q_{H_2}^{S_4O_6}$ amounted to only about 30 % of the $-Q_{H_2}^{SO_4}$.

Dithionite. Four mol. hydrogen were taken up/ion of dithionite added, a result consistent with the view that the ion decomposes primarily to sulphate and sulphur, the first of which is reduced. However, sulphide-formation was consistently in excess of 1 mol. S'' /ion $\text{S}_2\text{O}_4^{''}$ required by the equation:

though insufficient for the equation:

(see Table 4). Since freshly-prepared solutions of the specimen smelled of H_2S it is possible that the discrepancy was due to sulphide impurities. If

this was the case, however, the values obtained for the hydrogen uptake must be regarded as excessive. The question was not studied further since dithionite is most unstable and very difficult to obtain pure.

Metabisulphite. Potassium metabisulphite was reduced straight-forwardly in the Warburg manometer with the uptake of 6 mol. $\text{H}_2/\text{ion S}_2\text{O}_5^{2-}$ and with the formation of 2 mol. sulphide (see Table 4):

Sulphur. No hydrogen uptake in excess of the blank was detected in the Warburg manometer using 're-precipitated' or re-distilled sulphur (10^{-5} g-atom/flask). Sulphur added as a colloidal sol containing polythionates (0.0194% $\text{H}_2\text{S}_5\text{O}_6$) showed at first a slow hydrogen uptake ($-\text{Q}_{\text{H}_2} = 20 \text{ mm}^3/\text{mg.}/\text{hr.}$) in excess of the blank and its ultra-filtrate ($-\text{Q}_{\text{H}_2} = 7 \text{ mm}^3/\text{mg.}/\text{hr.}$). To see whether this was a genuine reduction of sulphur the flasks were shaken for 16 hr. in all, by which time the $-\text{Q}_{\text{H}_2}$ in all vessels was almost zero. The total amounts of hydrogen absorbed in the flasks containing sulphur corresponded to reduction of the sulphur present, and proportionate amounts of sulphide were found (Table 5). Traces of sulphide were also found in the flasks containing the ultra-filtrate of colloidal sulphur, and a small amount of hydrogen had been absorbed, but this can reasonably be ascribed to polythionate impurities known to be present in the sulphur sol.

Table 5. *Sulphide formed and hydrogen absorbed by resting suspensions of D. desulphuricans (Hildenborough) with colloidal sulphur and its ultra-filtrate*

Substrate	Amount added ($\mu\text{mol.}$)	Hydrogen absorbed ($\mu\text{mol.}$)	Sulphide formed ($\mu\text{mol.}$)
Sodium sulphate	2.5	10.1 (10)	2.54 (2.5)
Colloidal sulphur	4.66	5.30 (4.66)	5.05 (4.66)
Ultra-filtrate	*	0.62 (0)	0.203 (0)
No addition	—	0.40 (0)	0 (0)

Theoretical figures given in brackets.

* A volume of ultra-filtrate equivalent to $4.66 \mu\text{mol.}$ of sulphur in the unfiltered sol was added.

Other compounds. The following compounds were tested and were not reduced by resting cell suspensions: sodium dithionate, sodium formaldehyde sulphonylate, ammonium perdisulphate, ammonium sulphamate, sodium methanesulphonate, sodium β -hydroxyethanesulphonate, sodium ethylsulphate, and dimethylsulphone. In view of the growth-promoting action of cysteine (Report, 1950; Postgate, 1951), cystine (British Drug Houses Ltd.) was tested in the Warburg manometer but was not reduced.

DISCUSSION

D. desulphuricans strain 'Hildenborough' was able to utilize sulphate, thio-sulphate, sulphite and tetrathionate for growth and its resting metabolism.

It was also apparently able to use dithionite and metabisulphite, but it is likely that these compounds decompose spontaneously to sulphate and sulphite respectively before use. The organism was unable to utilize dimethylsulphone or several sulphur-containing oxy-acid ions, including two aliphatic and one aromatic sulphonates.

This and other strains of *D. desulphuricans* were unable to utilize elementary sulphur purified by re-distillation, and it is unlikely that this failure to grow was due to an allotropic change in the sulphur during purification since *Th. thio-oxidans* could use it for growth. Nor is it likely that an adaptive change in the bacteria is needed for sulphur utilization, since the four strains tested in autotrophic culture were first grown with impure sulphur—a condition likely to favour such an adaptation. No sign was obtained of growth-inhibitory impurities in the purified sulphur, since the 'blank' growth and hydrogen uptake was not prevented by these preparations. It is, therefore, likely that the activity of some sulphur preparations is due to the presence of oxidation products as impurities, and this view is favoured by the fact that a purified specimen of sulphur regained its activity after prolonged standing in air. In his original work on this subject Baars (1930) used colloidal sulphur prepared by the action of excess H_2S on SO_2 , but this method also yields polythionates other than dithionate, which could have been responsible for the activity observed. Baars ensured the absence of sulphate and sulphite in his preparations, but did not check them for polythionates. In the present work, a sulphur sol prepared by a similar method was active, and since its ultra-filtrate was relatively inactive, the effect of the sol was probably not due to polythionates. In Baar's work, the yields of sulphide obtained from colloidal sulphur did not depend on the amounts of sulphur added, but in this work a direct quantitative relationship, between hydrogen absorbed, sulphide formed and colloidal sulphur added was found, which constitutes further evidence for the direct reduction of colloidal sulphur.

D. desulphuricans was able to use tetrathionate in place of sulphate, but unfortunately specimens of pure pentathionate and trithionate were not available for testing. The organism did not use dithionate, a result consistent with the theoretical view that this ion is not of the same structural type as the higher polythionates (Ephraim, 1934).

The mechanism of sulphate reduction. These studies raise some interesting points concerning the mechanism of the reduction of sulphate. The main substances found to be reducible were sulphate, sulphite, thiosulphate, tetrathionate and colloidal sulphur, dithionite and metabisulphite being of secondary importance since they probably acted by spontaneous decomposition to one of the other ions. It is unlikely that sulphite, thiosulphate or tetrathionate underwent a preliminary decomposition to sulphate since the hydrogen uptakes and sulphide yields corresponded quantitatively to the reduction of the unchanged substrate: a preliminary decomposition would decrease the sulphide yield by formation of inactive sulphur, as may occur in the case of dithionite, and a preliminary oxidation to sulphate would cause considerably increased hydrogen uptakes to be found. Thus there is no doubt

that these substances, as well as colloidal sulphur, are directly reduced by the organism, but it does not follow that they are necessarily intermediate in the normal reduction of sulphate. Since the sulphite ion requires three molecules of hydrogen for its reduction, as compared with four hydrogen molecules required by sulphate, the fact that the $-Q_{H_2}$ values were similar with these two compounds implies that the molar rate of sulphite utilization was greater than the molar rate of sulphate utilization by about 33 %. This suggests that sulphite is a normal intermediate in sulphate reduction, and this view is supported by the report (Postgate, 1949) that sulphite will overcome the inhibition of sulphate reduction by sodium selenate in a non-competitive manner. On the other hand, it is unlikely that thiosulphate and tetrathionate are intermediates in the normal reduction process since the specific rates at which these two compounds are reduced are less than those for sulphate and sulphite. These rates have been measured as the $-Q_{H_2}$ values for various substrates, and, whereas the $-Q_{H_2}^{SO_4}$ and $-Q_{H_2}^{SO_3}$ were usually similar for a given cell suspension, the $-Q_{H_2}^{S_2O_3}$ was lower by about 40 % and the $-Q_{H_2}^{S_4O_6}$ by about 60 %. Similarly, colloidal sulphur is probably not a normal intermediate since the $-Q_{H_2}^S$ amounted to only about 10 % of the $-Q_{H_2}^{SO_3}$. The conclusion that thiosulphate and tetrathionate are not normal intermediates in sulphate-reduction is not surprising on theoretical grounds. Thiosulphate and tetrathionate are both less oxidized than sulphite, so they would presumably come at stages beyond sulphite in a scheme such as:

But the scheme as written above involves only divalent ions containing one sulphur atom, and for the appearance of ions containing more sulphur atoms a loss of electrons from the system must take place, e.g.:

This is a formal oxidation, but is nevertheless not an impossible reaction, and one could picture a series of oxidative polymerizations occurring, coupled with reductions, which would lead to sulphide by way of polythio-ions. However, not only would electrons be continually lost in this process, but a logical result would be the formation of free sulphur or a very complex polythio-ion. Moreover, for the stages which can be represented as:

a considerable absorption of electrons by the system would be required as the converse of the oxidative polymerization stages.

Given suitable balance of the oxidative and reductive stages, hypotheses on these lines representing the mechanism of sulphate-reduction are not out of the question, but it is simpler to regard the normal process as:

and to regard the reduction of thiosulphate and tetrathionate as an independent process which would probably be written as:

Even so, there is no evidence from the present work that thiosulphate is an intermediate in tetrathionate reduction, and it is possible that the two compounds are reduced by independent mechanisms.

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