

# Tetrathionate Reduction and Production of Hydrogen Sulfide from Thiosulfate

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INTRODUCTION .....	192
REDUCTIONS OF SULFUR COMPOUNDS PERFORMED BY NON-SULFATE-REDUCING BACTERIA .....	193
OCCURRENCE OF TETRATHIONATE AND THIOSULFATE REDUCTION .....	193
Reactions under Consideration .....	193
Natural Occurrence of the Substrates .....	194
Survey of Bacteria That Reduce Tetrathionate and Thiosulfate .....	194
H <sub>2</sub> S from Thiosulfate versus H <sub>2</sub> S from Cysteine.....	195
Source of the confusion .....	195
Different volatile sulfides.....	195
Different sources of H <sub>2</sub> S.....	196
TETRATHIONATE AND THIOSULFATE REDUCTION BY THE SULFATE-REDUCING BACTERIA .....	196
TETRATHIONATE AND THIOSULFATE REDUCTION BY <i>ENTEROBACTERIACEAE</i> .....	197
<i>Citrobacter, Proteus, and Salmonella</i> .....	197
Background .....	197
Reductases.....	197
Electron transport system .....	198
Regulation .....	198
Genetics .....	199
Assimilatory thiosulfate reduction .....	199
Other <i>Enterobacteriaceae</i> .....	199
TETRATHIONATE AND THIOSULFATE REDUCTION BY THIOSULFATE-OXIDIZING BACTERIA .....	199
<i>Thiobacillus</i> -Like Isolates .....	199
<i>Pseudomonas aeruginosa</i> .....	200
CONCLUDING REMARKS .....	200
LITERATURE CITED.....	201

## INTRODUCTION

In the biological sulfur cycle as presented in most textbooks of microbiology, reduced free sulfur (hydrogen sulfide) is produced in two different ways. It is the product of sulfate reduction by a unique group of anaerobic bacteria that includes *Desulfovibrio* and *Desulfotomaculum*, and it is released from organic matter without any change in oxidation state during processes of protein decomposition by a variety of other microorganisms. Although many organisms assimilate sulfate and reduce it for their biosynthetic needs, dissimilatory sulfate reduction appears to be performed exclusively by the sulfate-reducing bacteria (130). They obtain energy by means of an anaerobic respiration in which sulfate serves as the primary electron acceptor, although they can also use partially oxidized sulfur compounds for their energy needs (127, 130). These bacteria are typically found in mud or waterlogged soil that contains organic matter and oxidized sulfur (138). When hydrogen sulfide is found in other environments, it is generally assumed to be the product of protein decomposition.

On the other hand, it has long been known in the diagnostic laboratory that there are a number of bacteria that reduce thiosulfate or sulfite to H<sub>2</sub>S or reduce tetrathionate to thiosulfate independently of their biosynthetic needs. There are, in fact, about 10 different commercial media available for testing H<sub>2</sub>S production, and the ability to produce H<sub>2</sub>S is a valued taxonomic character. Most of the organisms that perform these reductions are quite familiar to us in many respects, yet we know very little about the biochemistry and physiology of their sulfur reductions or their contribution to the biological sulfur cycle.

The goal of this review has been to assemble and analyze the literature concerning tetrathionate and thiosulfate reductions by bacteria outside the sulfate-reducing group in the hopes of providing a foundation for future studies of these processes. The reduction of sulfur compounds by the well-characterized sulfate-reducing bacteria has been extensively studied and reviewed (83, 118, 119, 127-130, 138, 153, 161), and it was not our original intention to include these organisms in this review except as the point of departure for discussions of the comparable processes in other bacteria. However, in the reviews of the sulfate-reducing bacteria, thiosulfate and tetrathionate reductions are clearly periph-

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TABLE 1.  $E_0'$  values for redox couples associated with sulfur-containing electron acceptors and other electron carriers

Redox couple	$E_0'$ (mV)	Reference
$O_2/H_2O$	+818	153
$NO_3^-/NO_2^-$	+433	153
DMSO/DMS <sup>a</sup>	+160	179
TMAO/TMA <sup>b</sup>	+130	58
$S_4O_6^{2-}/S_2O_3^{2-}$	+170	63
	+80	79
	+24	153
Fumarate/succinate	+33	153
$SO_3^{2-}/S^{2-}$	-116	153
$S/S^{2-}$	-270	153
NAD/NADH	-320	153
$S_2O_3^{2-}/S^{2-} + SO_3^{2-}$	-402	153
	-420	58
$H^+/H_2$	-414	153
$CO_2/formate$	-432	153
$SO_4^{2-}/SO_3^{2-}$	-480	58
	-516	153

<sup>a</sup> DMSO/DMS, Dimethyl sulfoxide/dimethyl sulfide.

<sup>b</sup> TMAO/TMA, Trimethylamine oxide/trimethylamine.

eral subjects. Thus, to bring the point of departure into focus, it was necessary to include a brief discussion of tetrathionate and thiosulfate reductions performed by the sulfate-reducing bacteria.

#### REDUCTIONS OF SULFUR COMPOUNDS PERFORMED BY NON-SULFATE-REDUCING BACTERIA

The sulfur compounds that have been shown to be reduced by bacteria outside the sulfate-reducing group include tetrathionate, thiosulfate, sulfite, sulfur, and dimethyl sulfoxide. The  $E_0'$  values of the redox couples associated with these reductions are shown in Table 1 along with the values for certain other couples of interest to this discussion. Many of the bacteria found to reduce one or more of the above compounds have been tested for ability to reduce sulfate, and the results have always been negative (28, 136, 149, 150). This is not surprising in light of the low redox for the sulfate/sulfite couple, which not only necessitates very reduced growth conditions but also creates a significant energy deficit prior to the energy-yielding reactions. Thus, sulfate reduction offers little advantage to bacteria that can use other electron acceptors.

There are conflicting reports regarding the redox potential for tetrathionate reduction, but the reported values are all within a range near the fumarate/succinate couple at one end and near the trimethylamine oxide/trimethylamine couple at the other end. Oxidative phosphorylation can be facilitated by both trimethylamine oxide reduction (10, 94) and fumarate reduction (96). Thus, one might expect tetrathionate to serve as an electron acceptor for oxidative phosphorylation in the bacteria capable of reducing it to thiosulfate. Thiosulfate reduction, in contrast, does not appear to be a likely candidate for an anaerobic respiration that is accompanied by oxidative phosphorylation. The low redox for thiosulfate reduction and the fact that the ability to reduce thiosulfate is rarely found in the absence of the ability to reduce tetrathionate as well (as will be discussed) suggest that the significance of thiosulfate to energy metabolism in many bacteria may be closely tied to the role of tetrathionate.

The reduction of dimethyl sulfoxide appears to be part of the anaerobic respiratory repertoire of many bacteria (5, 137, 184, 185), but it is not performed by *Desulfovibrio* spp. (184). It has been studied in the photosynthetic bacteria (10, 94, 137, 182) and in *Escherichia coli* (12, 13). Dimethyl sulfoxide reduction is not related to the reactions associated with  $H_2S$  production. Instead, it bears more resemblance to trimethylamine oxide reduction (10, 94, 137). Thus, it is not within the scope of this review.

The reduction of sulfite is the key energy-yielding reaction of the sulfate-reducing bacteria (6, 102). However, it is performed by very few bacteria outside of this group even though many bacteria can reduce sulfite in assimilatory processes (118, 139). Dissimilatory sulfite reduction has been reported for certain clostridial species (44, 49, 78), a free-living *Campylobacter*-like isolate (77), and, among the *Enterobacteriaceae*, *Salmonella* spp. and *Edwardsiella tarda* (117; M. A. Clark and E. L. Barrett, submitted for publication). Sulfite and thiosulfate reductions are closely associated reactions in the sulfate-reducing bacteria (138). Information regarding dissimilatory sulfite reduction by facultative anaerobes is still too fragmentary for meaningful discussion at this time.

The reduction of sulfur to sulfide seems to be confined to very special situations. It is performed by some sulfate-reducing bacteria (11, 38), and it appears to be an important energy-yielding process for *Desulfuromonas acetoxidans*, an anaerobic acetate-oxidizing species found in the same environments as the sulfate-reducing bacteria (120). Sulfur also serves as an electron acceptor for the same *Campylobacter*-like isolate noted above to reduce sulfite (77), for "*Spirillum* sp. strain 5175" (177), an isolate which resembles the *Campylobacter*-like isolate (73), for *Wolinella succinogenes* (90), and for several thermophilic archaeobacteria (143). Elemental sulfur is also reduced by *Beggiatoa* spp., thereby facilitating survival during short periods of exposure to anaerobic conditions (101). None of the facultative anaerobes that reduce thiosulfate or tetrathionate have been reported to reduce elemental sulfur.

#### OCCURRENCE OF TETRATHIONATE AND THIOSULFATE REDUCTION

##### Reactions under Consideration

Tetrathionate and thiosulfate reductions are associated with reductases that perform the following reactions: Tetrathionate reductase— $2[H] + S_4O_6^{2-} = 2S_2O_3^{2-} + 2H^+$  (66, 123, 124); Thiosulfate reductase— $2[H] + S_2O_3^{2-} = H_2S + SO_3^{2-}$  (50, 51, 114). As the first reaction predicts, tetrathionate reduction results in significant acidification of the medium (66, 69, 123). In fact, typically, the reduction in vitro is linear for only a short period because the reductase loses activity as the pH falls (66, 69, 121). Medium acidification during tetrathionate reduction is the basis of simple qualitative tests for the occurrence of this activity or its mutational loss in tetrathionate reducers (85). The earliest assays described for tetrathionate reduction consisted of iodometric titrations of the thiosulfate formed in the presence of physiological electron donors (123). Tetrathionate reductase has also been assayed by measuring hydrogen consumption in an assay mixture containing hydrogenase and benzyl viologen or methyl viologen as electron carrier (51, 87, 121) or by measuring benzyl viologen oxidation by tetrathionate (34). It is somewhat surprising that all of these

assays, including titrations of thiosulfate, are possible in crude extracts of bacteria capable of thiosulfate reduction as well as tetrathionate reduction, but the investigators report that thiosulfate reduction in vitro does not begin until all tetrathionate has been reduced (66, 124).

Thiosulfate reductase activity is distinct from the activity of rhodanase (thiosulfate:cyanide sulfurtransferase; EC 2.8.1.1.), an enzyme present in mammalian cells and many

microorganisms, which cleaves thiosulfate nonreductively into sulfur and sulfite (170). *Desulfotomaculum nigrificans* has been shown to possess a rhodanase activity that is separate from the thiosulfate reductase protein (24). Thiosulfate reduction cannot be assayed with benzyl viologen because the  $E_0'$  for the thiosulfate couple is lower than that for benzyl viologen. However, it can be assayed by measuring methyl viologen oxidation (7) or by measuring hydrogen consumption with methyl viologen as a carrier (33, 56).

TABLE 2. Tetrathionate and thiosulfate reductions and H<sub>2</sub>S production by the *Enterobacteriaceae* and selected representatives of other families<sup>a</sup>

Organism	Tetra- thionate reduction <sup>b</sup>	Thiosulfate reduction		
		H <sub>2</sub> S in TSI <sup>c</sup>	Other reports <sup>d</sup>	
			Reaction	Assay (reference) <sup>e</sup>
<i>Escherichia coli</i>	-	-	+	LAS (28, 149)
<i>Shigella</i> spp.	-	-	-	LAS (28, 149)
<i>Salmonella typhi</i>	+	+	-	LAS (149)
			+	LAS (136)
<i>S. paratyphi</i> A	-	-	-	LAS (28, 149)
<i>S. gallinarum</i>	-	+	-	TSI (117)
<i>S. typhimurium</i> and most other sero- types	+	+	+	LAS (28)
<i>Citrobacter freundii</i>	+	+	+	LAS (149)
<i>C. diversus</i>	-	-	Some +	PIA (18)
<i>C. amalonatica</i>	+	-	NF	
<i>Klebsiella pneumoniae</i>	-	-	NF	
<i>Enterobacter</i> spp.	-	-	NF	
<i>Erwinia</i> spp.	-	-	NF	
<i>Serratia marcescens</i>	Most +	-	-	H <sub>2</sub> S chemical assay (150)
<i>S. liquefaciens</i>	+	-	NF	
All other <i>Serratia</i> spp.	-	-	NF	
<i>Hafnia alvei</i>	Some +	-	+	PIA and LAA (39, 48)
<i>Edwardsiella tarda</i>	+	+	+	PIA (73)
<i>Proteus mirabilis</i> and <i>P. vulgaris</i>	+	+	+	LAS (28, 150)
<i>Providencia</i> spp.	+	-	Some +	LAS (28)
<i>Morganella</i> spp.	+	-	Some +	LAS (28)
<i>Yersinia pestis</i>	-	-	NF	
<i>Y. enterocolitica</i>	Most -	-	NF	
<i>Vibrio cholerae</i>	-	-	NF	
<i>V. parahaemolyticus</i>	+	-	-	LAA (165)
<i>Aeromonas hydrophila</i>	Some +	-	-	AH (62)
<i>Pasteurella multocida</i>	+	+	NF	
<i>Alteromonas putrefaciens</i>	+	-	+	PIA (89)

<sup>a</sup> Genera included are those for which both tetrathionate reduction and thiosulfate reduction have been reported.

<sup>b</sup> Tetrathionate reduction reported by Richard (131), LeMinor and co-workers (86, 87), or Papavassiliou et al. (116).

<sup>c</sup> Reaction in triple sugar iron agar (TSI) as reported in *Bergey's Manual* (20, 73).

<sup>d</sup> Original reports of thiosulfate reduction which contradict *Bergey's Manual* or which involved tests other than triple sugar iron agar. NF, None found.

<sup>e</sup> Abbreviations: LAS, lead acetate strips; LAA, lead acetate agar (Difco Laboratories); PIA, peptone iron agar (Difco); AH, *Aeromonas hydrophila* medium (62).

#### Natural Occurrence of the Substrates

Thiosulfate and tetrathionate are reported to be present in most soils except in very humid regions (142). It has been suggested that heterotrophs are important in sulfur transformations in the drier regions of the world because dry soil is too aerobic for the sulfate-reducing bacteria (142). Thiosulfate could easily be produced from the sulfur added as fertilizer by means of chemical reaction with oxygen (160). Thiosulfate has also been found in significant quantities in certain marine environments, particularly in aerobic/anaerobic interfaces where the oxygen content is high enough to oxidize sulfide to thiosulfate, but too low for rapid chemical thiosulfate oxidation (164). Thiosulfate, being a by-product of mammalian cysteine catabolism, is also present in urine (136).

Tetrathionate is an unstable compound (69) and thus would not be expected to persist in soils. Its occurrence in soils may, instead, reflect the existence of natural processes of thiosulfate oxidation, either biological (140, 141, 160, 162-164, 166) or chemical (142). Thiosulfate is readily oxidized to tetrathionate; in fact, a tetrathionate solution can be prepared by mixing a thiosulfate solution with either iodine (69) or ferric chloride (85). Thus, any environment that contains thiosulfate is potentially an environment suitable for tetrathionate reduction.

#### Survey of Bacteria That Reduce Tetrathionate and Thiosulfate

The bacteria outside of the sulfate reducers which have been tested for both tetrathionate and thiosulfate reduction are compiled in Table 2. The reported results for bacteria tested for either tetrathionate reduction or H<sub>2</sub>S production (but not both) appear in Table 3. The data compiled in Table 2 show that the ability to produce H<sub>2</sub>S from thiosulfate is closely associated with the ability to reduce tetrathionate. There are only three reports of H<sub>2</sub>S production from thiosulfate in a bacterial group shown to be negative for tetrathionate reduction, and two of these reports were contradicted by other investigators. In contrast, the clear majority of the bacteria tested were either positive for both reductions or negative for both reductions, and about one-third were reported positive for tetrathionate reduction only. Perhaps this distribution indicates that the ability to reduce thiosulfate is of little value in the absence of the ability to reduce tetrathionate. A additional feature revealed in Table 2 is that there are contradictory reports concerning the ability to produce H<sub>2</sub>S from thiosulfate, a problem that stems from confusion regarding the source of reduced sulfur when bacteria are tested diagnostically for the "ability to produce H<sub>2</sub>S." The reasons for the confusion are the topic of the next section. The practical result of the confusion is that it is impossible to assemble an accurate list of bacteria that reduce thiosulfate. Undoubtedly, many of the H<sub>2</sub>S producers listed in Table 3 are bacteria that reduce thiosulfate to

TABLE 3. Organisms<sup>a</sup> tested for tetrathionate reduction or H<sub>2</sub>S production

Tetrathionate reduction <sup>b</sup>		H <sub>2</sub> S production <sup>c</sup>	
Positive	Negative	Positive	Negative
<i>Alcaligenes odorans</i>	<i>Acinetobacter</i>	( <i>Actinomyces</i> )	<i>Acetobacter</i>
( <i>A. faecalis</i> )	<i>Corynebacterium diphtheriae</i>	<i>Arachnia</i>	<i>Actinobacillus</i>
( <i>A. denitrificans</i> )	( <i>Flavobacterium</i> )	<i>Azotobacter</i> <sup>d</sup>	<i>Agrobacterium</i>
<i>Pseudomonas aeruginosa</i>	<i>Moraxella</i>	<i>Bacillus larvae</i> (45)	<i>Bifidobacterium</i>
<i>P. acidovorans</i>	<i>Pseudomonas cepacia</i>	<i>Bacterionema matruchotii</i>	<i>Bradyrhizobium</i>
( <i>P. maltophilia</i> )	<i>P. diminuta</i>	( <i>Bacteroides</i> ) (156)	<i>Brucella melitensis</i>
<i>Veilonella</i> (180)	<i>P. fluorescens</i>	( <i>Brucella abortus</i> )	<i>B. ovis</i>
<i>Xanthobacter</i> (73)	<i>P. picketti</i>	<i>B. neotomae</i>	<i>Capnocytophaga</i>
	( <i>P. putida</i> )	<i>B. ovis</i> (148)	<i>Cardiobacterium</i>
	<i>P. pseudoalcaligenes</i>	<i>Budvicia</i> (15) <sup>e</sup>	<i>Cedeceae</i>
	( <i>P. stutzeri</i> )	<i>Campylobacter</i> <sup>e</sup>	<i>Gardnerella</i>
	<i>Staphylococcus</i> (123)	<i>Clostridium</i> , many species	<i>Gluconobacter</i>
	<i>Streptococcus</i> (123)	<i>Francisella</i>	<i>Haemophilus</i>
	<i>Xanthomonas</i>	<i>Halobacterium</i>	<i>Halomonas</i>
		<i>Halococcus</i> <sup>d</sup>	<i>Kluyvera</i>
		<i>Kingella indologenes</i>	<i>Obesumbacterium</i>
		( <i>Lactobacillus plantarum</i> ) (82)	<i>Paracoccus</i>
		<i>Neisseria gonorrhoeae</i> (35) <sup>d</sup>	<i>Rahnella</i>
		<i>Pseudomonas mephitica</i> (104)	<i>Tatumella</i>
		( <i>Rhizobium meliloti</i> )	<i>Xanthobacter</i>
		<i>Rothia dentocariosa</i>	<i>Xenorhabdus</i>
		<i>Zymomonas</i> (46)	

<sup>a</sup> Genera included are those for which either tetrathionate reduction or H<sub>2</sub>S production (but not both) has been reported. Genus name only is given when property is characteristic of all species tested; species reported to differ are listed separately. Names in parentheses indicate that property was not characteristic of 100% of the isolates tested.

<sup>b</sup> Unless otherwise indicated, properties were reported by Richard (131).

<sup>c</sup> Unless otherwise indicated, properties were reported in *Bergey's Manual* (20, 73).

<sup>d</sup> Test was specific for the production of H<sub>2</sub>S from thiosulfate.

<sup>e</sup> Organisms were tested in triple sugar iron agar. In the case of *Campylobacter*, *C. sputorum* and *C. concisus* were positive in triple sugar iron agar, while *C. jejuni*, *C. coli*, and *C. fetus* were positive with lead acetate but not in triple sugar iron agar.

H<sub>2</sub>S, but many may also be putrefactive bacteria incapable of thiosulfate reduction. As discussed below, one must also question some of the results compiled in Table 2 even when the authors specified thiosulfate as the source of the H<sub>2</sub>S. The problem is that the source of reduced sulfur constituting a positive test for H<sub>2</sub>S is not as easy to establish as one might think.

#### H<sub>2</sub>S from Thiosulfate versus H<sub>2</sub>S from Cysteine

**Source of the confusion.** There are at least four problems underlying confusion in the interpretation of tests for H<sub>2</sub>S production. First, typical diagnostic testing uses media composed of varying amounts of thiosulfate and peptones. Second, peptones themselves contain varying amounts of sulfur amino acids and partially oxidized sulfur compounds (132, 155). Third, the typical diagnostic media also frequently contain fermentable carbohydrates, and H<sub>2</sub>S production from thiosulfate can be "masked" during sugar fermentation (21, 22) either because it is repressed (M. A. Clark and E. L. Barrett, submitted for publication) or because it cannot be detected (21, 22). Surprisingly, carbohydrates do not interfere with the detection of H<sub>2</sub>S from sulfite; in fact, fermentable carbohydrates are essential in media designed to detect sulfite, as contrasted with thiosulfate, reduction (117, 176). Finally, different results are obtained depending on the indicator system used. Precipitation of a dark or brightly colored sulfide is the most common indicator reaction. Thus, media for H<sub>2</sub>S testing may contain lead, iron, or bismuth or may be used with a strip of paper impregnated with a lead salt or 5,5'-dithiobis-2-nitrobenzoic

acid suspended above the culture. Although the detection mechanisms are similar in principle, in fact the results of the tests vary considerably with the choice of indicator. As discussed below, the contradictory results may actually underlie important differences in the origin and nature of the sulfide produced.

**Different volatile sulfides.** The 5,5'-dithiobis-2-nitrobenzoic acid strips were shown by McMeekin et al. (95) to detect a variety of volatile sulfides including methanethiol, dimethyl sulfide, and dimethyl disulfide in addition to H<sub>2</sub>S. For this reason, they were described as being "more sensitive" than lead assay strips, which detect only H<sub>2</sub>S (95). However, Rodler and co-workers, who investigated H<sub>2</sub>S detection with lead acetate and iron chloride (132, 133), showed that lead acetate will react with various thiols as well as with H<sub>2</sub>S. They reported (133) that many bacteria generally regarded as H<sub>2</sub>S<sup>-</sup> can give a positive "H<sub>2</sub>S test" in the presence of cysteine when lead acetate was used as the indicator, although most of them were negative when iron chloride was used in place of lead acetate. Rodler et al. attributed the positive test with lead acetate to the volatile sulfides released through the action of decarboxylases or deaminases on cysteine and proposed that iron chloride was, in contrast, specific for H<sub>2</sub>S. Other investigators have suggested, instead, that iron chloride was simply less sensitive than lead acetate strips (21, 28, 39, 44, 82, 165, 186), but Rodler et al. (132) reported that iron chloride detected H<sub>2</sub>S produced from thiosulfate sooner than did lead acetate. Unfortunately, the literature contains many contradictions regarding the ability to produce H<sub>2</sub>S even among investigators who all used lead acetate. A possible explanation for the contradic-

tions is that, although lead acetate reacts with many sulfides, it is most sensitive to  $H_2S$ , and the definition of positive versus negative results depends on whether or not a true  $H_2S$  producer is used as the positive control against which to judge the weaker reactions. The concentration of cysteine in the test medium is also a factor; bacteria generally regarded as  $H_2S^+$  yield a positive reaction with a far lower cysteine concentration than required to obtain a positive test for bacteria generally regarded as  $H_2S^-$  (28, 109). There are fewer contradictions among investigators who used iron chloride as the indicator, but there are also bacteria that are consistently negative in such tests that have been shown to produce  $H_2S$  from cysteine in biochemical assays, as will be discussed below.

**Different sources of  $H_2S$ .** Many investigators have attempted to distinguish between the detection of  $H_2S$  from cysteine, i.e., cysteine desulfhydrase activity, and  $H_2S$  from thiosulfate, i.e., thiosulfate reductase activity (62, 97, 149–151). Cysteine desulfhydrase catalyzes the formation of  $H_2S$ , ammonia, and pyruvate from cysteine, and it is found in many organisms (30, 47, 72). It is induced by cysteine (30, 97, 151) and is thought to protect against cysteine toxicity (30). In *Salmonella* spp., induction requires cysteine concentrations of  $>0.1$  mM, and the enzyme is repressed by sulfide at concentrations of  $>0.1$  mM (30). The enzyme in *Proteus* spp. was shown to be much more active under aerobic conditions than under anaerobic conditions (151). Thiosulfate reduction, in contrast, is favored under anaerobic or microaerophilic conditions (33, 34, 62, 89, 104).

In the testing of bacteria for the ability to reduce thiosulfate, it is essential to differentiate  $H_2S$  produced through thiosulfate reduction from  $H_2S$  produced via cysteine degradation. It is often assumed that  $H_2S$  detected in complex media lacking added thiosulfate constitutes a test for cysteine degradation, and many bacteria tested in such media are specified as " $H_2S^+$  from cysteine" in the current *Bergey's Manual* (73). However the protein digests in complex media can contain thiosulfate (132, 155). Furthermore, one cannot assume that the enzymatic production of  $H_2S$  from cysteine is necessarily independent of the pathway for thiosulfate reduction. In fact, the ability to reduce thiosulfate strongly influences the results of tests for  $H_2S$  from cysteine. For example, both *Salmonella typhimurium* ( $H_2S^+$  from thiosulfate) and *E. coli* ( $H_2S^-$  from thiosulfate) contain cysteine desulfhydrase (45), but only *S. typhimurium* appears  $H_2S^+$  in a medium consisting of gelatin, nutrient broth, and iron chloride (74, 80). Similarly, *Proteus vulgaris* ( $H_2S^+$  from thiosulfate) and *Serratia marcescens* ( $H_2S^-$  from thiosulfate) were shown to produce equal amounts of  $H_2S$  from cysteine in assays (150), but only *P. vulgaris* appears  $H_2S^+$  in a medium containing peptone, cysteine, and iron chloride (133). It is interesting that Tilley, in 1923, recommended thiosulfate as an ingredient for testing  $H_2S$  production not because its reduction represented a taxonomic characteristic distinct from the production of  $H_2S$  from cysteine, but because it "improved" the distinction between  $H_2S^+$  and  $H_2S^-$  species (154, 155).

Both *E. coli* and *Serratia* spp. do give positive tests in the peptone-plus-cysteine medium if lead acetate rather than iron chloride is used as the indicator (133). One possible explanation for the failure to detect the  $H_2S$  with iron salts is that a reduction of the iron is a prerequisite for a positive reaction with iron chloride and only the species that can produce  $H_2S$  from thiosulfate are capable of reducing the iron. When iron is used, it is usually present as a ferric salt, either because it was added in that form or because the

ferrous salt was autoclaved in a neutral medium. A positive test for  $H_2S$  based on the formation of iron sulfide, i.e., ferrous sulfide, requires both the reduction of ferric iron and sulfide precipitation. In contrast, the lead in lead acetate does not need to be reduced. We have found that *E. coli* does appear  $H_2S^+$  in the gelatin medium if filter-sterilized ferrous chloride is added to the autoclaved medium (M. A. Clark and E. L. Barrett, unpublished data).

#### TETRATHIONATE AND THIOSULFATE REDUCTION BY THE SULFATE-REDUCING BACTERIA

Much less is known about thiosulfate and tetrathionate reduction by the sulfate-reducing bacteria than is known about their reduction of sulfate and sulfite. Postgate (125) was the first to report that thiosulfate and tetrathionate could serve as electron acceptors for sulfate-reducing bacteria. He demonstrated that a resting cell suspension of *Desulfovibrio desulfuricans* (a strain now designated as *Desulfovibrio vulgaris*) quantitatively reduced thiosulfate or tetrathionate to sulfide with molecular hydrogen as reductant (125). That sulfide was produced from both compounds indicates that the reduction of tetrathionate and thiosulfate constitutes a tributary of sulfite reduction. Later it was shown that lactate also served as an in vivo electron donor (57). In a cell-free extract, thiosulfate reduction with  $H_2$  proceeded very slowly in the absence of the artificial electron carrier methyl viologen (56). Similarly, it has since been shown that the purified thiosulfate reductases from *D. gigas* and *Desulfotomaculum nigrificans* do not reduce thiosulfate to sulfide when reduced nicotinamide adenine dinucleotide (NADH), NADPH, reduced glutathione, or cysteine is provided as electron donor in place of reduced methyl viologen (51, 100).

Thiosulfate reductase has been purified from *D. vulgaris* (4, 50), *D. gigas* (51), and *Desulfotomaculum nigrificans* (100). The enzyme from *D. gigas* also catalyzes the reduction of tetrathionate to thiosulfate, using benzyl viologen as electron donor (51), but tetrathionate reductase as such has not been purified from this organism or any other sulfate-reducing bacteria. The thiosulfate reductases from all three organisms were found in the soluble fraction (4, 50, 51, 100, 107), and the enzyme has been shown in *D. vulgaris* to be functionally localized on the cytoplasmic side of the cytoplasmic membrane (7). The molecular weight of the thiosulfate reductase from *D. vulgaris* (Miyazaki F strain) (4) was 87,000, and that from *D. gigas* was determined to be 220,000 (51). The optimum pH was generally in the slightly alkaline range: it was 7.2 to 7.9 for the *D. vulgaris* (Miyazaki F) enzyme (4), 8.0 to 9.0 for the *D. vulgaris* (Hildenborough) enzyme (50), and 7.4 to 8.0 for the *D. gigas* enzyme. The enzyme from *Desulfotomaculum nigrificans* was reported to function equally well at all pH values from 6.0 to 8.0. The  $K_m$  for thiosulfate was determined for the *D. gigas* (0.5 mM) and *Desulfotomaculum nigrificans* (130 mM) enzymes. The surprisingly high latter value suggests that thiosulfate reduction is not a primary pathway for energy metabolism in this organism.

Electron transport from  $H_2$  to thiosulfate has been investigated in several species of *Desulfovibrio*. Cytochrome  $c_3$  (molecular weight, 13,000) can function as the sole electron carrier between the hydrogenase and thiosulfate reductase activities in *D. vulgaris* (4, 126) and *D. desulfuricans* (19). Studies with purified hydrogenase and thiosulfate reductase from *D. gigas* showed that the most efficient electron transport system (other than methyl viologen) consisted of both

cytochromes  $c_3$  and  $cc_3$  (molecular weight, 26,000) (51). Ferredoxin and flavodoxin both strongly stimulated thiosulfate reduction in cell-free extracts of *D. gigas* (52; L. Guarraia, E. J. Laishley, N. Forget, and H. D. Peck, Jr., *Bacteriol. Proc.*, p. 133, 1968), but neither appeared to be involved in thiosulfate reduction by *D. vulgaris* (4, 181).

Thiosulfate reductase has been proposed to be part of an enzyme system for a three-step reduction of sulfite in which trithionate and thiosulfate are intermediates (42, 43, 147), although there is now convincing evidence that thiosulfate is only a by-product of sulfite reduction when sulfite is in excess (60) and free intermediates are not normally produced during the reduction of sulfite to sulfide (27). It is unlikely that thiosulfate reduction alone supports growth of adenosine triphosphate formation because the growth yields for *D. vulgaris* on  $H_2$  with thiosulfate are equivalent to yields obtained with sulfite (6, 102). This finding suggests, instead, that thiosulfate is simply a source of sulfite.

Thiosulfate has also been shown to facilitate growth on lactate by *D. baculatus* (135), *D. thermophilus* (134), *D. africanus* (59), *Desulfotomaculum ruminis* (29), and *Thermodesulfobacterium commune* (183). The enzyme systems involved have not been examined. Among these organisms, only *D. africanus* was tested for growth with tetrathionate and was found negative (59). Thiosulfate also supports the growth of *Desulfobulbus propionicus* on propionate (175) and *Desulfobacter postgatei* on acetate (152, 173). In contrast, *Desulfotomaculum acetoxidans* grows only with sulfate as electron acceptor (174). In their examination of 92 isolates of sporulating and nonsporulating sulfate-reducing bacteria, Skyring et al. (139) found that most strains grew on lactate plus thiosulfate. Thus, thiosulfate reduction appears to be a common adjunct to the energy-yielding systems of the sulfate-reducing bacteria.

#### TETRATHIONATE AND THIOSULFATE REDUCTION BY ENTEROBACTERIACEAE

##### *Citrobacter*, *Proteus*, and *Salmonella*

**Background.** *Citrobacter*, *Proteus*, and *Salmonella* are the major  $H_2S$ -producing and tetrathionate-reducing genera of the *Enterobacteriaceae*. Reports of  $H_2S$  production by *Proteus* and *Salmonella* spp. go back into the clinical literature of the 19th century (see reference 136 for discussion of early observations). Studies of  $H_2S$  production in *Proteus* spp. by Tarr in 1933 (150, 151) and later by Mitsuhashi and Matsu in 1953 (97) demonstrated that there were two mechanisms of  $H_2S$  production, one associated with thiosulfate reduction and the other involving only cysteine. Tarr (150) pointed out that many bacteria were capable of the latter type of  $H_2S$  production, while thiosulfate reduction was a more limited property. Mitsuhashi and Matsu (97) reported that formate was a major electron donor for thiosulfate reduction, while it did not have much effect on the production of  $H_2S$  from cysteine, and that the two substrates induced specifically the respective enzyme systems for  $H_2S$  production. More than 30 years elapsed before any further studies of thiosulfate reduction by *Enterobacteriaceae* were published except in the context of studies focused on tetrathionate reduction or on anaerobic respiratory systems that happen to share some features with thiosulfate reduction.

Tetrathionate reduction was first described in 1942 by

Pollack et al. (124), who were investigating the selective action of tetrathionate in selective media for *Salmonella* spp. They showed that tetrathionate reduction was a stoichiometric conversion of tetrathionate into thiosulfate (123) which was dependent on an electron donor such as a sugar or formate (123) and could be induced by tetrathionate in whole-cell preparations (70). They also found that tetrathionate improved yields of *Salmonella* spp. growing in complex media (68, 69) and suggested that tetrathionate was serving as an electron acceptor (68, 69, 124). Interest in tetrathionate reduction was apparently not contagious as more than 20 years elapsed before any further reports were published. The renewed interest was sparked by the possible usefulness of tetrathionate reduction as a taxonomic character that was easily tested (85–87, 116) and by the work of LeMinor, Pichinoty, and co-workers (88, 121), who showed that the tetrathionate reduction shared many features with nitrate reduction. About the same time, Stouthamer and co-workers began a study of tetrathionate and thiosulfate reduction in *Proteus*, while Kaprálek and co-workers examined tetrathionate reduction in *Citrobacter freundii*. The combined work of the latter two groups of investigators constitutes the vast majority of the literature concerning tetrathionate and thiosulfate reduction by the *Enterobacteriaceae*.

**Reductases.** Tetrathionate reductase was shown to be membrane bound in both *Proteus mirabilis* (112, 113) and *C. freundii* (105). The enzyme has been partially purified from *P. mirabilis* and shown to have a molecular weight of 133,000 and two subunits of molecular weights 43,000 and 90,000 (112, 113). The enzyme activity in *C. freundii* has been partially characterized (66, 106, 121). The  $K_m$  for tetrathionate was 2 mM (121), and the pH optimum for tetrathionate reduction was 8.0 (66, 105), with a 50% drop in activity when the pH drops below 7.2 (105). This is somewhat surprising in light of the acidification that accompanies the reduction of tetrathionate to thiosulfate (66, 69, 123). Possibly the concentrations of tetrathionate ordinarily encountered or handled by these bacteria is typically low or the environments in which tetrathionate is found are well buffered. The former possibility is supported by the observation that tetrathionate reductase in *C. freundii* is inhibited by concentrations of tetrathionate of  $>5$  mM (66).

A striking feature of the purified enzyme from *P. mirabilis* is its catalytic versatility; it was found to catalyze, in addition to tetrathionate reduction, thiosulfate and trithionate reduction as well as sulfide oxidation (113). Reductase function by the enzyme purified from *P. mirabilis* appears to be tightly regulated, as thiosulfate reduction was reported not to begin until all tetrathionate had been reduced (112, 113). This may explain why the thiosulfate formed from tetrathionate was not reduced by whole cells of *C. freundii* (63, 66) or *Salmonella* spp. (123, 124). Similarly, it has been shown that the concentration of tetrathionate in tetrathionate broth determines whether or not *Salmonella* spp. will produce  $H_2S$  during growth; if the concentration exceeds 30 mM, acidification kills the cells before the tetrathionate is consumed and thiosulfate reduction never commences (69). Oltmann and Stouthamer (113) showed that the thiosulfate formed from tetrathionate stimulated tetrathionate reduction rather than thiosulfate reduction by the purified enzyme.

Thiosulfate reduction by *P. vulgaris* has been shown to involve only the sulfane sulfur (93). In *S. heidelberg*, both sulfur atoms are reduced, but the sulfane sulfur is reduced entirely to  $H_2S$  before reduction of the sulfonate sulfur begins (93). Presumably, sulfonate sulfur reduction repre-

sents the activity of an anaerobic sulfite reductase; *S. typhimurium*, but not *P. vulgaris*, produces H<sub>2</sub>S from sulfite as well as thiosulfate (Clark and Barrett, submitted). The presence of the latter activity may explain why *S. typhimurium* mutants defective in the biosynthetic reduction of sulfite can grow without cysteine under anaerobic conditions, while they behave as cysteine auxotrophs under aerobic conditions (9).

Oxygen was shown to interfere with tetrathionate reduction by extracts of *C. freundii* (65, 121). Kaprálek and Pichinoty (65) attributed the effect to the preferential transfer of electrons to oxygen rather than to a direct effect of oxygen on the enzyme because cyanide prevented the inhibition, and the inhibition was reversible.

The tetrathionate/thiosulfate reductases are membrane bound (105, 112, 121). Studies of tetrathionate reduction by a number of *Enterobacteriaceae* have shown that this activity, like nitrate and trimethylamine oxide reductions, is absent in pleiotropic chlorate-resistant mutants defective in the processing of molybdenum (32, 33, 75, 88, 111, 145; D. L. Riggs and E. L. Barrett, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, K246, p. 218). Molybdenum has been demonstrated in tetrathionate reductase from *P. mirabilis* (110) and *S. typhimurium* (53).

**Electron transport system.** Although coupling of oxidative phosphorylation to tetrathionate reduction has not been demonstrated directly, growth yield studies suggest that it does occur (63, 68, 146). Kaprálek (63) calculated that 1.3 mol of adenosine triphosphate could be formed per mol of tetrathionate reduced. Stouthamer and Bettenhausen (146) reported a P/2e ratio of 0.2 for tetrathionate reduction in *P. mirabilis*. Thiosulfate, in contrast, was found not to improve anaerobic growth yields of *P. mirabilis* (146). However, the results with thiosulfate are difficult to evaluate in light of the fact that the growth medium contained glucose, which has been shown to repress thiosulfate reductase in *S. typhimurium* (Clark and Barrett, submitted).

Formate, hydrogen, and NADH have all been shown to serve as electron donors for tetrathionate reduction (71, 105, 106, 114, 123). Novotný and Kaprálek (106) reported that succinate, lactate, malate, and glycerol were, in contrast, not efficient electron donors for in vitro tetrathionate reduction by *C. freundii*, although Stouthamer and Bettenhausen (146) reported that lactate and glycerol can support anaerobic growth of *P. mirabilis* in the presence of tetrathionate. Formate is the only donor that has been shown to reduce thiosulfate (71). The pathway by which formate reduces thiosulfate may be different from that associated with tetrathionate reduction because hydrogen gas is evolved simultaneously with thiosulfate reduction, while no gas is evolved during reduction of tetrathionate by formate (71).

Both Novotný and Kaprálek (106) and Stouthamer and co-workers (34, 114) reported that extracts of cells induced for tetrathionate reductase contain type *b* cytochromes which can be reduced with formate and partially reoxidized by tetrathionate. de Groot and Stouthamer (34) found that thiosulfate also reoxidized the same *b* cytochromes. When reduced, the *Citrobacter* cytochrome had an absorbance peak at 560 nm at 20°C (106). Two peaks were found in formate-reduced extracts of *P. mirabilis*: 561 and 563.5 nm at 15°C. On the other hand, an essential role for cytochromes in tetrathionate or thiosulfate reduction has not been demonstrated and is actually contradicted by other findings. In *P. mirabilis*, tetrathionate oxidized only 15% of the type *b* cytochromes reduced by formate (33). Furthermore, on

media designed to indicate tetrathionate reduction (85), *Salmonella* heme mutants formed colonies indistinguishable from wild-type colonies (Clark and Barrett, unpublished data). The heme mutants, however, did lack the ability to reduce thiosulfate to H<sub>2</sub>S as well as methyl viologen-linked thiosulfate reductase (Clark and Barrett, submitted), which could indicate that thiosulfate reductase is a heme-containing enzyme or that cytochromes are necessary for thiosulfate reduction.

Quinones were implicated in electron transfer to tetrathionate in *C. freundii* (106), but were not identified. The *Citrobacter* tetrathionate reductase has also been shown to accept electrons from reduced flavin mononucleotide, but not from NADH or NADPH (121). *S. typhimurium* menaquinone mutants fail to reduce thiosulfate (75, 76), but produce almost as much acid on the tetrathionate test media (85) as does the wild type (Clark and Barrett, unpublished data), which suggests that menaquinones are necessary for thiosulfate, but not tetrathionate, reduction. Ubiquinone mutants resembled the wild type with respect to both thiosulfate and tetrathionate reduction (Clark and Barrett, unpublished data); thus, ubiquinones are probably not involved in electron transport to tetrathionate and thiosulfate by *S. typhimurium*. Clearly, the information available at this time regarding electron carriers is too incomplete to postulate an electron transport scheme for tetrathionate or thiosulfate reduction.

**Regulation.** Several aspects of the regulation of tetrathionate reduction are consistent with an overall picture of respiratory control in which there is a hierarchy of electron transport pathways, each of which represses all systems with lower energy potential and is, similarly, repressed during operation of the higher-energy-potential systems. In accordance with this picture, neither tetrathionate nor thiosulfate reduction occurs in the presence of oxygen (33, 65, 112, 123, 145) or nitrate (2, 34). Oxygen has also been shown to prevent expression of  $\beta$ -galactosidase activity in Mu d1(*lac*) insertion mutants affected in the production of H<sub>2</sub>S from thiosulfate (3; Clark and Barrett, submitted). Furthermore, tetrathionate reduction prevents hydrogen evolution via the formate hydrogenlyase system (33, 34, 71, 122), while thiosulfate reduction, a reaction of very low redox potential, can occur simultaneously with hydrogen evolution (34, 71). Oxygen per se is reported to repress the synthesis of tetrathionate reductase (33, 34, 65), but it does not inhibit enzyme activity; however, electron transport to oxygen reversibly inhibits the activity in vitro (65, 121). On the other hand, both inhibition and repression by nitrate occur only when nitrate respiration is possible (2, 34, 145). In *P. mirabilis* it has been shown that tetrathionate reductase subunits are still present in the membrane during repression by nitrate respiration, but they are not active (111). Similarly, formate hydrogenlyase has been found in extracts of tetrathionate-grown cells, even though no hydrogen was being produced.

There are conflicting reports regarding the induction of tetrathionate and thiosulfate reductases by their substrates. Both activities are reported to be constitutive in *P. mirabilis* grown under anaerobic conditions in the absence of nitrate (33), while tetrathionate is reported to induce tetrathionate reductase in anaerobic cultures of *Citrobacter* (121) and *Salmonella* (123, 124) species. A variety of reduced sulfur compounds, including thiosulfate, will induce thiosulfate reductase activity in *S. typhimurium* growing anaerobically in minimal medium (Clark and Barrett, submitted). Thiosulfate reductase activity in anaerobic cultures of *C. freundii* is

undetectable (66, 121) even though *C. freundii* do produce hydrogen sulfide from thiosulfate (73). A major difficulty in resolving the conflicting reports is that many of the early studies used media containing glucose, and both reductases are subject to catabolite repression (64; Clark and Barrett, submitted). Catabolite repression of thiosulfate reductase in *S. typhimurium* is quite severe; 5.6 mM glucose abolishes all enzyme activity in cells grown in minimal medium (Clark and Barrett, submitted). Glucose repression of tetrathionate reductase in *Citrobacter* is somewhat less pronounced; activities in glucose-grown cells are about 15% of those in succinate-grown cells.

**Genetics.** Very little is known about the genetics of thiosulfate and tetrathionate reduction. Casse and co-workers (25) isolated a *Salmonella* mutant defective specifically in tetrathionate reduction and mapped the mutation. The mutant was not examined further. Voll and co-workers (167, 168) identified a gene essential for the production of H<sub>2</sub>S from thiosulfate in the vicinity of the *his* operon. They showed that this gene could be expressed in *E. coli*, although *E. coli* strains carrying this gene on an F' episome produced less H<sub>2</sub>S than did the *Salmonella* parent (167). They found that *E. coli* strains with extended deletions in the vicinity of *his* did not produce H<sub>2</sub>S when the episome from *S. typhimurium* was introduced, suggesting that there are genes in the same region present in both *E. coli* and *S. typhimurium* which are required for H<sub>2</sub>S production (167).

**Assimilatory thiosulfate reduction.** That both *S. typhimurium* and *E. coli* assimilate thiosulfate (37, 84, 99) while only *S. typhimurium* produces H<sub>2</sub>S from thiosulfate suggests that, in *Salmonella* sp., the dissimilatory and assimilatory reductions of thiosulfate are distinct. However, there are some differences between the two organisms regarding thiosulfate assimilation which may provide clues regarding possible common components of the assimilatory and dissimilatory reactions in *Salmonella* spp. In *S. typhimurium*, thiosulfate assimilation involves the activity of *O*-acetylserine sulfhydrylase B. This enzyme catalyzes the formation of *S*-sulfo-cysteine from *O*-acetylserine and thiosulfate (98, 99). It is encoded by *cysM* (54, 99). Although the activity of this enzyme with sulfide, with which it synthesizes cysteine as opposed to *S*-sulfo-cysteine, is great enough to permit wild-type growth rates on sulfate (55), the primary *O*-acetylserine sulfhydrylase activity which functions in cysteine synthesis is that of *O*-acetylserine sulfhydrylase A (54, 55), the product of *cysK*. The *cysK* and *cysM* genes are located adjacent to and on opposite sides of *cysA*; *cysA* encodes sulfate permease, which also serves as the permease for thiosulfate (36, 108). Although CysK<sup>-</sup> CysM<sup>+</sup> mutants are prototrophs, CysK<sup>+</sup> CysM<sup>-</sup> mutants are prototrophic only aerobically and are bradytrophs under anaerobic conditions (40, 54). This suggests that *O*-acetylserine sulfhydrylase B is required for rapid cysteine synthesis under anaerobic conditions. Furthermore, although the various genes constituting the cysteine regulon, including *cysM*, require a functional *cysB* gene (the positive regulator) for induction under aerobic conditions, *cysB* mutants behave as prototrophs under anaerobic conditions (9). Thus far, only *O*-acetylserine sulfhydrylase A has been detected in *E. coli* (41, 172). Aerobic/anaerobic differences in cysteine requirement have not been noted in *E. coli*. It will be interesting to see whether the aerobic/anaerobic differences in cysteine biosynthesis in *Salmonella* spp. and the existence of an enzyme for the specific incorporation of thiosulfate are actually reflections of the enzyme activities associated with dissimilatory thiosulfate reduction. As noted

below, the same question also emerges in the study of reductions performed by *Pseudomonas aeruginosa*.

#### Other Enterobacteriaceae

Hydrogen sulfide is also produced from thiosulfate by the newly described *Enterobacteriaceae* genus *Budvicia* (15) and by *Edwardsiella tarda* (73). *Edwardsiella tarda* also reduces tetrathionate (73), but there have been no studies of these reductions in either group. *Edwardsiella tarda* may be especially interesting because it is the only member of the *Enterobacteriaceae* family other than *Salmonella* that also produces H<sub>2</sub>S from sulfite (117).

Hydrogen sulfide-producing variants of *E. coli* have been isolated on numerous occasions (8, 16, 31, 80, 81, 91, 92, 115, 144, 169). In several cases, the ability to produce hydrogen sulfide was shown to be plasmid mediated (23, 80, 81, 91, 115, 144), although the nature of the plasmid differed from isolate to isolate. The plasmids also carried genes for raffinose utilization (23, 91, 115) in some cases and for tetracycline resistance (61, 144) in others. The plasmid guanine-plus-cytosine content was shown in one case to be about 40% (81) and in another case to be 50% (14), which suggests that the *E. coli* strains may have received the ability to produce hydrogen sulfide from diverse donors. One of the H<sub>2</sub>S<sup>+</sup> variants was tested for the ability to reduce tetrathionate and was found to be negative (61). In many of the reports, hydrogen sulfide production was tested in commercial triple sugar iron agar which contains 1% (wt/vol) lactose. This is interesting in light of the fact that lactose-positive *Salmonella* spp. appear H<sub>2</sub>S<sup>-</sup> in this medium, due to the "masking" of the H<sub>2</sub>S character by fermentation (22). Apparently, regulation of plasmid-mediated thiosulfate reduction in *E. coli* differs from the regulation observed in *Salmonella* spp.

There are also reports of variants of *Shigella sonnei* (1), *Klebsiella pneumoniae* (17), and an otherwise H<sub>2</sub>S<sup>-</sup> species of *Citrobacter diversus* (18), that reduce thiosulfate to H<sub>2</sub>S, but the isolates were not examined further. Trencavan et al. (157) warned that some of the reports of H<sub>2</sub>S production by strains of H<sub>2</sub>S<sup>-</sup> species might be mistaken; they showed that some "H<sub>2</sub>S<sup>+</sup>" *E. coli* isolates were actually mixed cultures containing the anaerobe *Eubacterium lentum*, which produces H<sub>2</sub>S and which can grow under semianaerobic conditions if cocultured with certain facultative anaerobes.

#### TETRATHIONATE AND THIOSULFATE REDUCTION BY THIOSULFATE-OXIDIZING BACTERIA

##### *Thiobacillus*-Like Isolates

The utilization of thiosulfate as an electron donor for aerobic growth is well documented among a number of bacteria and is generally associated with the genus *Thiobacillus*. Tetrathionate is also metabolized by many of the same organisms (67, 159), and some investigators have proposed that tetrathionate is an intermediate in the oxidation of thiosulfate (166), although there is also strong evidence to the contrary (67). Anaerobic tetrathionate metabolism of such organisms was first reported by Trudinger (159), who observed that brief anaerobic incubation "sparked" the aerobic oxidation of tetrathionate by *Thiobacillus* sp. strain X. His studies of the anaerobic metabolism of tetrathionate (159) and trithionate (158) suggested that the substrates were oxidized in the same way as under aerobic conditions and that the oxidation of these compounds was most active



under microaerophilic conditions. The interpretation of these studies is complicated by the nonenzymatic reactivity of tetrathionate under the conditions used (67).

Thiosulfate-oxidizing heterotrophs which actually reduce tetrathionate to thiosulfate under anaerobic conditions have since been isolated and examined. What is interesting about these studies is that they revealed the existence of reversible enzymes catalyzing both thiosulfate oxidation and tetrathionate reduction. Trudinger (160) described a "Thiobacillus-like" tetrathionate reducer isolated from soil. Because aerobic growth with thiosulfate resulted in the induction of the tetrathionate reductase, he suggested that the same enzyme might be responsible for both thiosulfate oxidase and tetrathionate reductase. He was unable to demonstrate a physiological role for either activity as thiosulfate did not improve aerobic yields and the organism would not grow anaerobically with tetrathionate as an electron acceptor.

More recently, Tuttle and Jannasch (162, 164) clearly demonstrated the existence of a reversible thiosulfate oxidase/tetrathionate reductase in "marine heterotroph 16B," a different isolate which was found to respond to both compounds. Strain 16B was isolated from an aerobic thiosulfate enrichment (164). Thiosulfate was shown to stimulate its aerobic growth (163), and both tetrathionate and thiosulfate were found to support anaerobic growth on pyruvate or lactate (164). Nitrate also supported anaerobic growth (164). A soluble constitutive thiosulfate oxidase was purified from aerobically grown cultures, and it was found to catalyze tetrathionate reduction as well (162, 171). The optimum pH for thiosulfate oxidation was 6.0 to 6.5 and that for tetrathionate reduction was 7.5. That the  $K_m$  for thiosulfate (0.15 mM) was lower than that for tetrathionate (about 4 mM) and that thiosulfate inhibited tetrathionate reduction suggested that the primary function of this enzyme was probably thiosulfate oxidation (162). Thiosulfate was found to stimulate the aerobic growth of this organism under certain conditions, although it did not seem to be a primary energy source (163).

No other tetrathionate reductase activity was found in aerobic cells, but anaerobic cultures grown with tetrathionate contained an additional tetrathionate reductase (162, 171). This anaerobically induced tetrathionate reductase was found in the membrane fraction (171). Oxygen reversibly inhibited its activity (162). Membrane preparations from induced cells also contained a type *b* cytochrome with absorption peaks at 414, 537, and 570 nm. A thiosulfate reductase activity was copurified with the inducible tetrathionate reductase (171), suggesting that, as with *Proteus* spp., the two reductions are carried out by the same enzyme.

#### *Pseudomonas aeruginosa*

*P. aeruginosa* is not usually classified among the thiosulfate-oxidizing bacteria, but Starkey reported in 1934 that it oxidized thiosulfate to tetrathionate in a manner similar to the "Trautwein" thiobacilli which had been described as *Thiobacillus*-like heterotrophs (140). In the same experiments, *Thiobacillus novellus* and *T. thioparus* oxidized thiosulfate to sulfate rather than to tetrathionate, indicating that the thiosulfate oxidations in *P. aeruginosa* and the other heterotrophs did not represent the same enzyme systems as were found in the autotrophs (141). *P. aeruginosa* is also capable of tetrathionate reduction (123). Although it has been reported to be  $H_2S^-$  from thiosulfate (149), it does

precipitate iron sulfide in several commercial media used to test  $H_2S$  production in the clinical laboratory (Barrett, unpublished data).

Chambers and Trudinger (26) demonstrated that *P. aeruginosa*, like *S. typhimurium*, can synthesize *S*-sulfocysteine from thiosulfate. It seems unlikely that the reaction is central to cysteine synthesis in *P. aeruginosa* because cysteine in the growth medium did not repress activity, and hydrolysis of *S*-sulfocysteine into cysteine was not detected. Extracts with *S*-sulfocysteine synthase activity were found to oxidize NADPH in the presence of reduced glutathione and *S*-sulfocysteine, a reaction which would be expected to yield cysteine and sulfite as products (26). This observation is very interesting because it suggests a mechanism for the dissimilatory reduction of thiosulfate to sulfide by *P. aeruginosa* or *S. typhimurium* in which cysteine (derived from thiosulfate by means of the *S*-sulfocysteine synthase) would be the source of sulfide released as  $H_2S$ . The involvement of cysteine as the substrate for the enzyme that releases  $H_2S$  during thiosulfate reduction might help to explain why thiosulfate-reducing bacteria produce  $H_2S$  from cysteine more consistently than do bacteria which contain only cysteine desulfhydrase (as was discussed above). The thiosulfate reducers would contain two enzymes which catalyze the formation of  $H_2S$  from cysteine. The existence of an enzyme in thiosulfate-reducing bacteria that releases  $H_2S$  from cysteine but is distinct from cysteine desulfhydrase would also help to explain the curious results of Guarneros and Ortega (47) regarding the stoichiometry of the cysteine desulfhydrase in extracts of *S. typhimurium*, namely, the production of more  $H_2S$  than pyruvate from cysteine.

*P. aeruginosa* is ideal for future studies of thiosulfate/tetrathionate reactions in the pseudomonads because its genetic system is well developed, and much is known about the biochemistry of its energy metabolism.

#### CONCLUDING REMARKS

Tetrathionate and thiosulfate reduction by the non-sulfate-reducing bacteria may be fundamentally different from the analogous reactions in the sulfate reducers. The latter bacteria reduce tetrathionate and thiosulfate completely to sulfide in a process associated with soluble enzymes and type *c* cytochromes that also participate in the reduction of sulfite. It would appear, then, that the reductions of tetrathionate to thiosulfate and thiosulfate to sulfite in the sulfate-reducing bacteria constitute a branch leading to the central energy-yielding reaction, namely, the reduction of sulfite. In contrast, among other microorganisms, the ability to reduce tetrathionate and thiosulfate is far more common than the ability to reduce sulfite. Furthermore, the reductases are membrane bound, and the cytochromes induced during tetrathionate and thiosulfate reduction are of the *b* type.

A role for tetrathionate reduction and thiosulfate reduction in the absence of sulfite reduction is somewhat difficult to imagine. The problem is that tetrathionate, although energetically favorable, is quite unstable, and thus likely to be rare, and thiosulfate, although more common, offers little in the way of energetic advantage, especially to organisms that can use other electron acceptors. Most of the bacteria that reduce tetrathionate also reduce nitrate. Many investigators who have studied tetrathionate reduction have pointed out similarities between the two reductions such as the common role of formate as electron donor (33, 71, 88), the common dependency on *chl* gene function for enzyme

biosynthesis (32, 33, 88, 110, 145), the common anaerobic expression (88, 114), and the possible common association with type *b* cytochromes (106, 111, 112). However, such similarities are not sufficient justification for stuffing tetrathionate and thiosulfate reduction into a mold that was shaped around nitrate reduction without considering other mechanisms and roles for tetrathionate and thiosulfate reduction based on the unique features of these two electron acceptors.

One striking unique feature is the ease with which thiosulfate and tetrathionate are interconverted and the catalytic versatility of the enzymes associated with their metabolism. Perhaps the metabolism of these compounds involves cyclic mechanisms which enable the cell to generate oxidizing or reducing power depending on cellular needs regarding regeneration of electron carriers or processing of nutrients such as metal cofactors. The extreme pH changes associated with the metabolism of tetrathionate might also be used to advantage in the manipulation of the proton gradient across the cell membrane.

A feature of thiosulfate reduction that is not characteristic of nitrate reduction is the production of an extremely toxic product, namely, H<sub>2</sub>S. Volatile sulfides have been shown to contribute to the pathogenicity of certain oral bacteria by rendering the mucosa more permeable (103, 156). Perhaps H<sub>2</sub>S production by the *Enterobacteriaceae* contributes to their ability to colonize tissues in other regions of the body.

Our knowledge of the mechanisms of H<sub>2</sub>S production by bacteria which do not reduce sulfate is still too scanty to postulate a role for these organisms in global sulfur transformations. However, the widespread occurrence of the ability to reduce tetrathionate and thiosulfate does suggest that these reductions are significant to the survival of many bacteria which do not rely on the sulfite produced for their energy needs.

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