Dimethyl Sulphoxide Reduction by Micro-organisms

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Dimethyl sulphoxide (DMSO) was reduced to dimethyl sulphide by a wide variety of micro-organisms, including prokaryotes and eukaryotes, aerobes and anaerobes. Dimethyl sulphone was not reduced by any of the organisms tested. Cell-free extracts of *Escherichia coli* reduced DMSO using reduced pyridine nucleotides as electron donors. Activity was greater in anaerobically grown cells than in those grown aerobically. Two other sulphoxides, methionine sulphoxide and tetramethylene sulphoxide, substantially inhibited DMSO reduction by extracts. Mutants of *E. coli*, which were unable to reduce biotin sulphoxide to biotin, were tested for their ability to reduce DMSO in whole cells and extracts. These mutants were in four different gene loci, *bisA* to *bisD*. DMSO reductase activity of the mutants was generally less than that of the wild-type strain, and activity depended upon the gene locus involved, the growth medium and the growth conditions. Only the *bisA* mutant had very low activity under all conditions. All of the *bis* mutants were able to grow using methionine sulphoxide as a sulphur source, indicating that biotin sulphoxide and methionine sulphoxide are reduced by different enzyme systems. DMSO may be reduced by both of these enzyme systems.

INTRODUCTION

Dimethyl sulphoxide (DMSO) is a waste product of paper mills which has recently received attention because of its potential medicinal value as a solvent which can be used to pass drugs through lipid membranes (Leake, 1967). It is also a possible intermediate in the global sulphur cycle as an atmospheric oxidation product of dimethyl sulphide (DMS) (Lovelock, Maggs & Rasmussen, 1972).

Sulphoxides are generally formed from sulphides by peroxidation (Snow, Finley & Kohler, 1976). Biotin sulphoxide and methionine sulphoxide are common metabolites (Black *et al.*, 1960; Melville, 1954; Sato *et al.*, 1958), and their reduction to sulphides by micro-organisms has been shown (Cleary & Dykhuizen, 1974; Sourkes & Trano, 1953). Certain pesticides are highly toxic to mammals when in the sulphoxide form, and microbial reduction may serve to detoxify them (Alexander, 1974). DMSO reduction to DMS has been shown in the cat (Distefano & Borgstedt, 1964), in the cow and other animals (Tiews *et al.*, 1975) and in plants (Smale, Lasater & Hunter, 1975). Ando *et al.* (1957) surveyed members of the Enterobacteriaceae and found that while all strains of *Escherichia coli* tested reduced DMSO, no strains of *Shigella* and only one out of 40 strains of *Salmonella* reduced it; they suggested that this reaction might be of diagnostic value.

Dykhuizen (1973) isolated several mutant strains, from a biotin-requiring parent strain of *E. coli*, which could not reduce biotin sulphoxide to biotin (*bis*). These mutants were in four gene loci, which were called *bisA*, *bisB*, *bisC* and *bisD*. Three of these genes, *bisA*, *bisB*

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and *bisD*, mapped very close to nitrate reductase genes. Methionine-requiring mutants which also could not reduce biotin sulphoxide could grow using methionine sulphoxide instead of methionine, indicating that the enzyme systems for reducing biotin sulphoxide and methionine sulphoxide are different. Cleary & Dykhuizen (1974) developed an enzyme assay for biotin sulphoxide reductase in cell-free extracts of *E. coli*, and demonstrated that the enzyme was present in the wild-type strain but not in any of the *bis* mutants.

In studies of the sulphur cycle, we found that DMSO was readily reduced to DMS by anaerobically incubated lake sediments and sewage sludge. To determine whether this reduction was biological, we surveyed cultures of several micro-organisms for DMSO reduction. We found that DMSO reduction was common in micro-organisms, and so went on to study the process more closely in *E. coli* using cell-free enzyme preparations and the mutants isolated by Dykhuizen (1973).

METHODS

Organisms. All cultures were obtained from the University of Wisconsin, Department of Bacteriology culture collection, except for *Desulfovibrio* sp., which we isolated from sewage sludge. *Escherichia coli* K12 Hfr Cavalli was obtained from Dr Martha Howe, Department of Bacteriology, University of Wisconsin. Mutants of *E. coli* K12 unable to reduce biotin sulphoxide (*bis*) were obtained from Dr A. del Campillo-Campbell, Department of Microbiology, Stanford University, and are more fully described in Table 4.

Culture media. Mineral salts W consisted of 1.0 g NH_4Cl , 0.13 g MgSO_4 , 3.0 g KH_2PO_4 and 6.0 g Na_2HPO_4 per l distilled water. *Escherichia coli* K12 was grown on mineral salts W plus 4.0 g glucose l^{-1} . The *bis* mutants and their parent strain were grown on GCAA medium, consisting of mineral salts W, plus 4.0 g glucose l^{-1} , 1.0 g Difco Casamino acids l^{-1} and 10^{-2} μg biotin ml^{-1} . TYEG medium consisted of 5.0 g Difco Bacto-tryptone, 5.0 g Difco yeast extract and 5.0 g glucose per l distilled water. For growth of *Clostridium butyricum*, 0.1 g sodium thioglycollate l^{-1} was added. *Desulfovibrio* sp. was grown on a lactate/sulphate mineral salts medium described elsewhere (Zinder & Brock, 1978). All cultures were incubated at 30 °C.

Gas chromatographic analysis of dimethyl sulphide. Dimethyl sulphide and other volatile sulphur compounds were measured using a Packard 419 gas chromatograph, fitted with a flame photometric detector. This detector is very sensitive to, and specific for sulphur compounds. The column used was similar to that of Stevens *et al.* (1971). The operating conditions were those described by Banwart & Bremner (1974). Dimethyl sulphide could also be identified readily by its strong characteristic odour.

DMSO reduction by laboratory cultures. To 10 ml TYEG medium sterilized in a 40 ml serum vial, sterile DMSO (sterilized by passage through a sterile 0.45 μm Millipore filter) was added to a final concentration of 100 μg ml^{-1} . After the medium had been inoculated, the vials were sealed with Teflon-coated rubber stoppers (Supelco). For anaerobic incubations, the headspace was gassed with sterile nitrogen purified by passage over heated copper filings. Cultures were incubated at 30 °C for 24 h, by which time they had all reached the stationary phase, and the headspace was then sampled and analysed for DMS by gas chromatography. Because DMS is insoluble in water, it passes rapidly and quantitatively into the headspace. Cultures of *Desulfovibrio* sp. were grown in anaerobic culture tubes on lactate/sulphate medium for 4 d at 30 °C.

DMSO reduction by crude extracts of *E. coli*. The procedure was based on that described by Cleary & Dykhuizen (1974) for biotin sulphoxide reduction. Cells were grown aerobically in shake flasks, and anaerobically in neoprene-stoppered bottles with nitrogen in the headspace. Early-stationary phase cells were harvested, washed three times, and resuspended in 1/200th the original volume of 50 mM-potassium phosphate buffer, pH 7.0, containing 2.0 mM-2-mercaptoethanol. The cells were broken in a French press at about 69 MPa (10000 lbf in^{-2}), and the extract was then dialysed overnight against phosphate buffer.

For the DMSO reduction assay, 0.8 ml of dialysed crude extract, containing 4 mg protein (Lowry *et al.*, 1951), was placed in a 7 ml serum vial. The vial was gassed with nitrogen purified by passage over heated copper filings, and sealed with an all-Teflon Mininert valve (Precision Sampling Co., Baton Rouge, Louisiana, U.S.A.). After incubation for 15 min in a 30 °C water bath, the reaction was started by adding 0.1 ml of 5 mM-NADH or 5 mM-NADPH and 0.1 ml of 10 mM-DMSO in rapid succession. The reaction mixture was incubated for 15 min at 30 °C and then a headspace sample was taken with a gas-tight syringe and analysed for DMS by gas chromatography. As previously stated, all DMS produced is found in the headspace.

Growth on methionine sulphoxide. For studies in which methionine sulphoxide was the sole sulphur source, mineral salts W was modified so that sulphate was replaced by chloride. Cells were grown in culture tubes containing the modified mineral salts W plus 20 mM-sodium lactate and 10^{-2} μg biotin ml^{-1} . Growth was measured as absorbance at 600 nm using a Spectronic 20 spectrophotometer (Bausch and Lomb).

Chemicals. Dimethyl sulphoxide and dimethyl sulphide were purchased from Fisher Chemical Co.,

Table 1. *Reduction of DMSO by various micro-organisms*

Cultures (10 ml) in TYEG growth medium containing 100 µg DMSO ml⁻¹ were incubated at 30 °C for 24 h in 40 ml serum vials sealed with Teflon-coated rubber stoppers. After incubation, DMS in the headspace was measured by gas chromatography. See Methods for full details.

Organism	Percentage of DMSO reduced to DMS	Organism	Percentage of DMSO reduced to DMS
<i>Escherichia coli</i>	18.0	<i>Bacillus subtilis</i>	17.0
<i>Salmonella typhimurium</i>	10.5	<i>Bacillus cereus</i>	0.5
<i>Klebsiella pneumoniae</i>	37.0	<i>Clostridium butyricum</i>	0.1
<i>Proteus vulgaris</i>	24.0	<i>Arthrobacter</i> sp.	5.0
<i>Providencia alcalifaciens</i>	26.0	<i>Desulfovibrio</i> sp.*	1.1
<i>Pseudomonas aeruginosa</i>	27.0	<i>Aspergillus niger</i>	3.0
<i>Staphylococcus aureus</i>	0.5	<i>Saccharomyces cerevisiae</i>	4.5
<i>Streptococcus faecalis</i>	0.2		

* Grown on lactate/sulphate medium for 4 d.

Fairlawn, New Jersey, U.S.A. L-Methionine, L-methionine-*dl*-sulphoxide, dimethyl sulphone, NADH and NADPH were obtained from Sigma. The methionine sulphoxide was shown to be free from contaminating methionine by thin-layer chromatography. Tetramethylene sulphoxide was purchased from Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A.

RESULTS

Survey of micro-organisms for DMSO reduction

A great variety of micro-organisms, including both prokaryotes and eukaryotes, reduced DMSO to DMS when growing on a complex medium (Table 1). To ensure that the DMS observed was derived from DMSO, controls to which no DMSO was added were examined. The amounts of DMS (and other volatile sulphur compounds, such as hydrogen sulphide, methane thiol and dimethyl disulphide) found in the controls was several orders of magnitude lower than in cultures with DMSO, and were most likely derived from breakdown of sulphur-containing amino acids (Kadota & Ishida, 1972). No volatile products other than DMS were formed from DMSO. Dimethyl sulphone was not reduced by any of the cultures.

Members of the Enterobacteriaceae and *Pseudomonas aeruginosa* and *Bacillus subtilis* caused the greatest DMSO reduction, while fermentative anaerobes, such as *Streptococcus faecalis* and *Clostridium butyricum*, reduced DMSO the least. Some DMSO was reduced by the *Desulfovibrio* sp. culture, but preliminary experiments have shown that DMSO and hydrogen sulphide react slowly to form DMS, so that the DMSO reduction observed may have been an artifact due to the great hydrogen sulphide production by *Desulfovibrio*. In other experiments (Zinder & Brock, 1978), we found that this strain of *Desulfovibrio* sp. could not grow using DMSO as an electron acceptor.

DMSO reduction by cell-free extracts of E. coli

Since *E. coli* was among those organisms which substantially reduced DMSO, and because this organism is so well characterized, the enzymic reduction of DMSO to DMS was studied using crude extracts of *E. coli* K12 grown in glucose/mineral salts medium. DMSO was reduced by these cell-free extracts in the presence of reduced pyridine nucleotides (Table 2). Activity was greater with NADH than with NADPH. If neither NADH nor NADPH was added, no activity was detected. Anaerobically grown cells had greater activity than aerobically grown cells, and the presence of DMSO in the growth medium did not substantially induce activity in anaerobically grown cells. If the extract had been boiled for 10 min, or if no extract was added, no activity was detected.

Table 2. *Reduction of DMSO by crude extracts of E. coli* K12

Assay mixtures (1 ml) contained extract (4 mg protein), 1 mM-DMSO and 0.5 mM-NADH or 0.5 mM-NADPH; they were incubated under nitrogen at 30 °C for 15 min.

Growth conditions	Electron donor	DMS produced [ng (mg protein) ⁻¹ min ⁻¹]
Anaerobic	NADH	31
Anaerobic	NADPH	11
Anaerobic	None	0.1
Anaerobic with 100 µg DMSO ml ⁻¹	NADH	34.5
Aerobic	NADH	12.5
Aerobic	NADPH	9.5
Anaerobic (boiled extract)	NADH	0.1
No extract	NADH	0
No extract	NADPH	0

Table 3. *Inhibition of reduction of DMSO by crude extracts of E. coli* K12

Conditions were as in Table 2.

Inhibitor	Inhibition (%)
Na ₂ S ₂ O ₃ (1 mM)	9
Na ₂ SO ₃ (1 mM)	4
Dimethyl sulphone (1 mM)	0
Methionine sulphoxide (1 mM)	61
Tetramethylene sulphoxide (1 mM)	74
NaNO ₃ (1 mM)	22
O ₂ (0.21 atm)	84
EDTA (10 mM)	39

Inhibitors of DMSO reduction by cell-free extracts of E. coli

Various compounds were tested to determine whether they inhibited DMSO reduction by crude extracts of anaerobically grown *E. coli* (Table 3). Sodium thiosulphate, sodium sulphite and dimethyl sulphone had little effect when added at a concentration equimolar with DMSO. The DMSO analogues, methionine sulphoxide and tetramethylene sulphoxide, substantially inhibited DMSO reduction, most likely by competition for an enzyme binding site. Sodium nitrate inhibited DMSO reduction, perhaps by funnelling electrons away, and oxygen prevented DMSO reduction by rapidly oxidizing the NADH (as determined by decrease of *A*₃₄₀). EDTA partially inhibited DMSO reduction, indicating that a complexed metal ion is involved.

DMSO reduction by cultures of bis mutants of E. coli

Mutants of *E. coli*, isolated by Dykhuizen (1973), which were unable to reduce biotin sulphoxide to biotin, were tested for their ability to reduce DMSO to DMS when growing on a complex medium (TYEG medium) or on a more defined medium (GCAA medium), similar to the one used by Cleary & Dykhuizen (1974). The effects of aerobic or anaerobic growth conditions in these media were also compared (Table 4).

In TYEG medium, the *bisC* mutant reduced as much DMSO as the wild-type strain, under both aerobic and anaerobic growth conditions. The *bisA*, *bisB* and *bisD* mutants showed little activity when grown aerobically, but there was a substantial increase in DMSO reduction by the *bisB* and *bisD* mutants when grown anaerobically. When grown aerobically at 37 °C, the *bisC* mutant reduced 58 % of the DMSO added, i.e. the same amount as at 30 °C, indicating that it is not a temperature-sensitive mutant growing at the permissive temperature.

Table 4. *Reduction of DMSO by the wild type and bis mutants of E. coli grown on TYEG or GCAA medium*

Conditions were as in Table 1. Results indicate the percentage of DMSO reduced to DMS.

Strain	Properties*	TYEG†		GCAA‡	
		Aerobic	Anaerobic	Aerobic	Anaerobic
R 879 (WT)	<i>bioA24</i>	48	64	7.2	70
R 876 (<i>bisA</i>)	<i>bioC18 chlE⁻ bisA18</i>	0.1	0.3	0.3	0.1
DD42 (<i>bisB</i>)	<i>bioA24 bisB</i>	0.1	4.5	1.5	9.8
DD133 (<i>bisC</i>)	<i>bioA24 bisC32</i>	58	88	7.0	74
DD39 (<i>bisD</i>)	<i>bioA24 bisD21 chlG⁻</i>	0.1	17	0.2	4.0

* From Dykhuizen (1973): *bio*, requires biotin; *bis*, unable to reduce biotin sulphoxide; *chl⁻*, chlorate resistant (unable to reduce nitrate).

† Tryptone yeast extract glucose medium.

‡ Glucose casamino acids medium.

Table 5. *Reduction of DMSO by crude extracts of the wild type and bis mutants of E. coli grown on GCAA medium*

Conditions were as described in Table 2. Results are expressed as ng DMS produced min⁻¹ (mg protein)⁻¹.

Strain	Aerobic		Anaerobic	
	NADH	NADPH	NADH	NADPH
R 879 (WT)	6.3	5.5	50	41
R 876 (<i>bisA</i>)	0.8	1.0	2.3	ND
DD42 (<i>bisB</i>)	3.7	3.9	18	ND
DD133 (<i>bisC</i>)	1.8	1.3	17	ND
DD39 (<i>bisD</i>)	1.6	1.6	9.0	ND

ND, Not determined.

When grown aerobically in GCAA medium, the wild-type strain and the *bisC* mutant again showed similar activity, but reduced considerably less DMSO than in the richer TYEG medium. This may be a function of the lower growth yield in GCAA medium rather than lower specific activity. However, when grown anaerobically, they both reduced substantially more DMSO. The *bisB* and *bisD* mutants showed low activity when grown aerobically, but reduced more DMSO anaerobically, as in TYEG medium. Under all growth conditions tested, the *bisA* mutant did not substantially reduce DMSO.

DMSO reduction by crude extracts of bis mutants of E. coli

Crude extracts of the biotin sulphoxide mutants were assayed for DMSO reduction to see whether enzyme levels paralleled the reduction found *in vivo*. Cells were grown aerobically or anaerobically in GCAA medium, and reduction by extracts was assayed in the presence of NADH or NADPH. As in *E. coli* K12, there was greater activity in the wild-type strain grown anaerobically than aerobically (Table 5). The increase in activity was similar to that found in growth media. NADH was slightly more effective as an electron donor than NADPH. The *bisA* mutant showed low activity under all conditions, as it did in the growth medium, and the *bisB* and *bisD* mutants showed an increase in activity when grown anaerobically, as they did in growth medium. The low activity of the *bisC* mutant is enigmatic, since its *in vivo* activity was similar to that of the wild-type strain.

Growth on methionine sulphoxide by bis mutants of E. coli

The ability of the *bis* mutants to grow using methionine sulphoxide as a sole sulphur source was examined to ascertain whether biotin sulphoxide and methionine sulphoxide reduction are independent reactions. Growth using methionine sulphoxide implies reduc-

Table 6. *Ability of bis mutants to grow using methionine sulfoxide as a sulphur source*Sulphur sources were provided at 10 $\mu\text{g S ml}^{-1}$. Results indicate turbidities, expressed as A_{600} .

Strain	Sulphur source			
	None	L-Methionine	L-Methionine sulfoxide	Sulphate
R 879 (WT)	0.07	0.31	0.29	0.90
R 876 (<i>bisA</i>)	0.11	0.30	0.25	ND
DD42 (<i>bisB</i>)	0.06	0.32	0.31	ND
DD133 (<i>bisC</i>)	0.11	0.31	0.33	ND
DD39 (<i>bisD</i>)	0.10	0.32	0.32	ND

ND, Not determined.

tion, since it is much more polar than methionine, and unlikely to be able to replace methionine in proteins.

Minimal growth occurred in sulphur-deficient medium (Table 6). Roberts *et al.* (1955) have determined that 10 $\mu\text{g S ml}^{-1}$ is sufficient for good growth of *E. coli*. All strains (with the possible exception of the *bisA* mutant) grew as well using methionine sulfoxide (10 $\mu\text{g S ml}^{-1}$) as a sulphur source as with methionine. Thus, all the mutants possess the ability to reduce methionine sulfoxide suggesting that there are two separate reduction systems for the two sulfoxides. As described by Roberts *et al.* (1955), growth yields were lower using methionine than sulphate as a sulphur source. DMSO could not serve as a sulphur source for growth.

DISCUSSION

DMSO is reduced to DMS by a wide variety of micro-organisms, both prokaryotic and eukaryotic. It is of interest that the two fermentative anaerobes included in the survey reduced DMSO the least. Obviously, a more comprehensive survey would have to be done before general conclusions can be made. Zinder & Brock (1978) have found that DMSO could be used as an electron acceptor for growth by a spiral organism, isolated from lake mud, and by *Proteus vulgaris*, but not by *E. coli*, *Pseudomonas aeruginosa* or *Desulfovibrio* sp. Although Ando *et al.* (1957) found that only one strain out of 40 *Salmonella* strains they tested reduced DMSO (as determined by formation of a precipitate with mercuric chloride), the one strain which we tested was able to reduce it significantly. Dimethyl sulphone was not reduced by any of the strains tested, which is not surprising since sulphones are very chemically and biochemically stable (Tonzetich, 1976). Dimethyl sulphone is a natural product, and humans excrete 4 to 11 mg per day in their urine (Williams, Burstein & Layne, 1966).

Escherichia coli has an enzyme system capable of reduction of DMSO to DMS using reduced pyridine nucleotides as electron donors. *In vitro* reduction is somewhat greater using NADH than NADPH. Anaerobically grown cells had greater activity than aerobically grown cells, and this could be due to either an increase in a pre-existing enzyme, or induction of a new one.

A reaction which is similar to DMSO reduction is the reduction of trimethylamine oxide by *Salmonella typhimurium*. Kim & Chang (1974) have found that a mutant without nitrate reductase was unable to reduce it. Our mutant studies have not given such clear results. Mutants unable to reduce biotin sulfoxide showed varying ability to reduce DMSO, but less activity than the wild-type strain, indicating a relationship between biotin sulfoxide and DMSO reductions. The *bisA* mutant showed consistently low DMSO reduction activity, both *in vivo* and *in vitro*. This may be due to the loss of some general component used by many reductases, since this mutant is also deficient in nitrate reductase. It should be mentioned that growth of the *bisA* and *bisD* mutants often lagged behind that of the other strains, indicating that some useful function was lost. The *bisB* and *bisD* mutants showed

increased DMSO reduction when grown anaerobically. It is not clear why the *bisC* mutant had identical activity with the wild-type strain *in vivo*, but less *in vitro*.

Although there appears to be some relationship between the system involved in the reduction of biotin sulfoxide and that which reduced DMSO, there are also some differences between them. Cleary & Dykhuizen (1974) found that NADPH rather than NADH was the better donor for biotin sulfoxide reduction. Also, none of the aerobically grown mutant strains had any appreciable biotin sulfoxide reductase activity compared with the wild-type strain, whereas the mutants other than *bisA* reduced up to 50% as much DMSO as did the wild-type strain.

The mutants (with the possible exception of *bisA*) could grow on methionine sulfoxide, in agreement with the findings of Dykhuizen (1973). Methionine sulfoxide inhibited *in vitro* DMSO reduction, which suggests that there is a separate system for reducing methionine sulfoxide, which is also capable of DMSO reduction. DMSO has greater structural similarity with methionine sulfoxide than with biotin sulfoxide, although both enzyme systems may reduce it.

It is unlikely that DMSO reduction is of any physiological benefit to *E. coli* or the other organisms shown to reduce it. Evidence for this is that *E. coli* cannot use DMSO as an electron acceptor for growth or as a sulphur source. Also DMSO in the growth medium does not induce greater synthesis of the enzyme system which reduces it. The activity we have found may be due to non-specific reduction by an enzyme system meant to reduce some other, dissimilar substrate. More likely, DMSO reduction is due to the presence of separate enzyme systems which reduce biotin and methionine sulfoxides, both of which are natural metabolites. Snow *et al.* (1976) suggested that a substantial portion of the methionine in foods is oxidized to the sulfoxide, and if this is so, it would be advantageous to an organism such as *E. coli*, living in the intestine, to reduce it. Methionine sulfoxide has a metabolic role in the absorption of methionine by the intestine (Sugawa, Akedo & Suda, 1960) and in pectin formation in plant tissue (Sato *et al.*, 1958), and it is quite possible that sulfoxides also play a role in microbial metabolism. Furthermore, if DMSO does play a role in the atmospheric sulphur cycle (Lovelock *et al.*, 1972), it is likely that any DMSO reaching active microbial communities would be reduced to DMS.

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REFERENCES

- ALEXANDER, M. (1974). Microbial formation of environmental pollutants. *Advances in Applied Microbiology* **18**, 1-73.
- ANDO, H., KUMAGAI, M., KARASHIMADA, T. & IIDA, H. (1957). Diagnostic use of dimethylsulfoxide reduction test within Enterobacteriaceae. *Japanese Journal of Microbiology* **1**, 335-338.
- BANWART, W. L. & BREMNER, J. M. (1974). Gas chromatographic identification of sulfur gases in soil atmospheres. *Soil Biology and Biochemistry* **6**, 113-115.
- BLACK, S., HARTE, E. M., HUDSON, B. & WARTOF-SKY, L. (1960). A specific enzymatic reduction of L(-)-methionine sulfoxide, and a related non-specific reduction of disulfides. *Journal of Biological Chemistry* **235**, 2910-2916.
- CLEARY, P. P. & DYKHUIZEN, D. (1974). Enzymatic reduction of D-biotin-*d*-sulfoxide with cell-free extracts of *Escherichia coli*. *Biochemical and Biophysical Research Communications* **56**, 629-634.
- DISTEFANO, V. & BORGSTEDT, H. H. (1964). Reduction of dimethylsulfoxide to dimethylsulfide in the cat. *Science* **144**, 1137-1138.
- DYKHUIZEN, D. (1973). Genetic analysis of the system that reduces biotin-*d*-sulfoxide in *Escherichia coli*. *Journal of Bacteriology* **115**, 662-667.
- KADOTA, H. & ISHIDA, Y. (1972). Production of volatile sulfur compounds by microorganisms. *Annual Review of Microbiology* **26**, 127-138.
- KIM, K. E. & CHANG, G. W. (1974). Trimethylamine oxide reduction by *Salmonella*. *Canadian Journal of Microbiology* **20**, 1745-1748.
- LEAKE, C. C. (1967). Introductory remarks. *Annals of the New York Academy of Sciences* **141**, 1-3.
- LOVELOCK, J. E., MAGGS, R. J. & RASMUSSEN, R. A. (1972). Atmospheric dimethyl sulphide and the natural sulphur cycle. *Nature* **237**, 452-453.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265-275.
- MELVILLE, D. B. (1954). Biotin sulfoxide. *Journal of Biological Chemistry* **208**, 495-501.
- ROBERTS, R. B., ABELSON, P. H., COWIE, D. B., BOLTON, E. T. & BRITTON, R. J. (1955). Studies of biosynthesis in *Escherichia coli*. *Carnegie Institute*

- of Washington Publication 607.
- SATO, C. S., BYERRUM, R. U., ALBERSHEIM, P. & BONNER, J. (1958). Metabolism of methionine and pectin esterification in a plant tissue. *Journal of Biological Chemistry* **233**, 138–141.
- SMALE, B. C., LASATER, M. J. & HUNTER, J. T. (1975). Fate and metabolism of DMSO in agricultural crops. *Annals of the New York Academy of Sciences* **243**, 228–236.
- SNOW, J. T., FINLEY, J. W. & KOHLER, G. O. (1976). A kinetic study of bisulfite reduction of methionine sulphoxide to methionine. *Journal of the Science of Food and Agriculture* **27**, 649–654.
- SOURKES, T. L. & TRANO, Y. (1953). Reduction of methionine sulfoxides by *Escherichia coli*. *Archives of Biochemistry and Biophysics* **42**, 321–326.
- STEVENS, R. K., MULIK, J. D., O'KEEFE, A. E. & KROST, K. J. (1971). Gas chromatography of reactive sulfur gases in air at the parts-per-billion level. *Analytical Chemistry* **43**, 827–831.
- SUGAWA, R., AKEDO, J. & SUDA, M. (1960). Intestinal absorption of amino acids. II. Isotopic studies on the amino acid absorption from the intestine using ^{35}S DL-methionine and ^{14}C DL-valine. *Journal of Biochemistry* **47**, 131–138.
- TIEWS, J., SCHARER, E., HARRE, N. & FLOGEL, L. (1975). Metabolism and excretion of dimethyl sulfoxide in cows and calves after topical and parenteral application. *Annals of the New York Academy of Sciences* **243**, 139–150.
- TONZETICH, J. (1976). Chromatographic separation of methionine, methionine sulphoxide, methionine sulphone, and their products of oral microbial metabolism. *Analytical Biochemistry* **73**, 290–300.
- WILLIAMS, K. I. H., EURSTEIN, S. H. & LAYNE, D. S. (1966). Dimethyl sulfone: isolation from human urine. *Archives of Biochemistry and Biophysics* **113**, 251–252.
- ZINDER, S. H. & BROCK, T. D. (1978). Dimethyl sulphoxide as an electron acceptor for anaerobic growth. *Archives of Microbiology* (in the Press).