Table 1. Amounts of immunoglobulin M and titers of cold agglutinins in the serum fractions of case A.

	IgM (mg/100 ml)	Cold agglutinin titer
Original serum	1350	8192
Supernatant*	1090	128
Cryoglobulin solution	110	4096
Cold agglutinin eluate	110	64

* Serum supernatant after separation of cryoglobulin.

cold agglutinin activity being in the cryoglobulin solution. The IgM concentration and cold-agglutinin titers of the various fractions from this patient are summarized in Table 1. The cryoglobulin solution contained the greatest cold agglutinin activity per milligram of IgM. The reason for differences in the ratios of cold agglutinin titer to IgM level in the various fractions is not yet known (*7*). In the Ouchterlony precipitation tests with the cryoglobulin solution and the isolated cold agglutinin, precipitin lines were obtained with antibody to IgM and antiserum to λ-chain only. No reaction was observed with antiserums against IgG, IgA, and κ-chains. The antiserums to κ-chain and λ-chain were each tested with Bence Jones proteins of type K and type L at concentrations of 0.2 mg/ml. Precipitin lines were obtained with homologous antigen and antibody only (Fig. 1). On account of the unexpected finding of λ-chains, the Ouchterlony precipitation tests were repeated with two different antierums to κ- and λ-chains (provided by Dr. D. Rowe); again precipitation with only the antiserum to λ occurred. The IgM in the cold agglutinin eluate was precipitable at 1°C.

Case B: A small quantity of serum was made available (by Dr. S. Worlledge) from a patient with the CHA syndrome in whom the cold agglutinin was of the third variety as defined above. There was no cryoglobulin in this serum which had a cold agglutinin titer of 256 at 4°C and an IgM concentration of 320 mg/100 ml. The isolated cold agglutinin which had an IgM concentration of 10 mg/100 ml and a cold agglutinin titer of 16 at 4°C was typed with specific antiserums as above. Precipitation lines exclusively with antiserum to λ and antiserum to IgM were obtained.

The serum of case C had a cold agglutinin titer of 16,000 with OI cells and a titer of 2 with Oi cells at 4°C; at 20°C the respective titers were 256 and less than 2; the antibody was therefore of I specificity; the serum IgM concentration was 920 mg/100 ml. The isolated cold agglutinin had an IgM concentration of 110 mg/100 ml and a cold agglutinin titer of 2048 at 4°C. In Ouchterlony precipitation tests of this eluate, precipitation lines were obtained only with antiserum to λ and to IgM (Fig. 1).

With Dr. Fahey's antiserums to κ- and λ-chains and antiserums against IgG, IgA, and IgM, 21 more cold agglutinins of I specificity in hemolytic anemia patients have been typed and found to be macroglobulins; κ-chains only were detected in all but one (three of them associated with *Mycoplasma pneumoniae* infection); both types of light chain were found in the remaining one, associated with *M. pneumoniae* (*8*). One of these is shown in Fig. 1. The findings in cases A, B, and C indicate that cold agglutinin activity can occur in the exclusive presence of λ-chains. The cold agglutinins in cases A and B were unusual in being of the third variety and there was the additional finding of cryoglobulin in case A. The cold agglutinin in case C, however, was entirely typical in showing specificity to I. It appears, therefore, that λ-chains in the absence of κ-chains can be associated not only with cold agglutinin activity but occasionally with specificity to I as well.

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9. I thank Dr. H. Davis for the opportunity of studying case A and Professor J. V. Dacie and Dr. S. Worlledge for the serums from cases B and C. I thank Dr. W. J. Jenkins and W. L. Marsh for the antibody specificity studies of the cold agglutinin from case A. For the specific antiserums to κ- and λ-chains, I thank Drs. J. Fahey and D. Rowe. Supported by grants from the Royal Free Hospital and the Medical Research Council (U.K.)

6 March 1967; revised 4 April 1967

Disulfur Monoxide: Production by Desulfovibrio

Abstract. Desulfovibrio desulfuricans *growing on agar surfaces produces a gas that appears to be identical to "Schenk's sulfur monoxide," which was later identified as disulfur monoxide. The gas stimulates surface growth of* Desulfovibrio *on an agar medium and is used by the cells as an electron donor for the reduction of benzyl viologen.*

Desulfovibrio desulfuricans (Midcontinent strain A) was found to produce a volatile substance or gas during cell growth of this organism on plates of trypticase soy broth plus 2 percent agar (*1*). This gas was characterized by its ability to stimulate growth of the same organism on agar plates of American Petroleum Institute (API) medium (*2*) where growth was ordinarily very poor, and also by its ability to be used by the organism as an electron donor for the reduction of benzyl viologen (BV). Violet (reduced) areas of the dye were produced in agar [2 percent agar plus 0.01*M* tris(hydroxymethyl)aminomethane-HCl buffer, *p*H 7.0 ± 1, and 0.01 percent BV] around heavy concentrations of *Desulfovibrio* cells when petri plates of the agar were placed in contact with this gas in a helium or nitrogen atmosphere.

Attempts to identify this gas by gas chromatography were unsuccessful. When the volatile material was removed—by flushing with helium—from a Brewer jar containing inoculated trypticase soy agar plates and was passed through a series of dry glass traps (cooled with a mixture of NaCl and ice, a mixture of methanol and dry ice, and liquid nitrogen, successively), the gas, as indicated by the two biological properties described above, could be concentrated in the liquid-nitrogen trap. An odor resembling that of H_2S was detected in the liquid-nitrogen trap as well as in the outlet tube from this trap, which was immersed in water. The water produced blackening of lead acetate paper. The odor of H_2S could be detected, however, only infrequently from a Brewer jar containing inoculated plates of trypticase soy broth plus agar. A sample of the gas from the trap in liquid nitrogn was analyzed in the mass spectrometer, but no sulfur compounds could be detected. Substances with mass peaks of 44, 28, and 16, corresponding to CO_2^-, N_2^-, or CO^- and CH_4^-, were detected. Car-

bon dioxide and methane were found to be metabolic products of *Desulfovibrio* (*3*). In that the trap, cooled with liquid nitrogen, was evacuated to remove helium before analysis, the compound might have been removed. In an attempt to isolate the sulfide compound, the biologically produced gas was passed (with helium as a carrier) through a series of three lead acetate traps (5 percent solution weight by volume) and then to the atmosphere by bubbling through water. No darkening in any of the three traps was noticed, but a strip of lead acetate paper placed in the water exposed to the air darkened in a minute or less. An odor resembling H_2S was also present. The gas appeared to form a sulfide only in contact with oxygen.

A small amount of black precipitate was obtained in a week by placing a lead acetate solution (5 percent weight by volume) in a petri plate on top of 12 inoculated plates of trypticase soy broth plus sodium lactate (4 ml per liter) plus agar in a Brewer jar (traces of oxygen present) with an atmosphere of hydrogen. The precipitate was collected, and the gas resulting from the addition of concentrated H_2SO_4 to the precipitate stimulated growth—in an atmosphere of nitrogen—on API agar

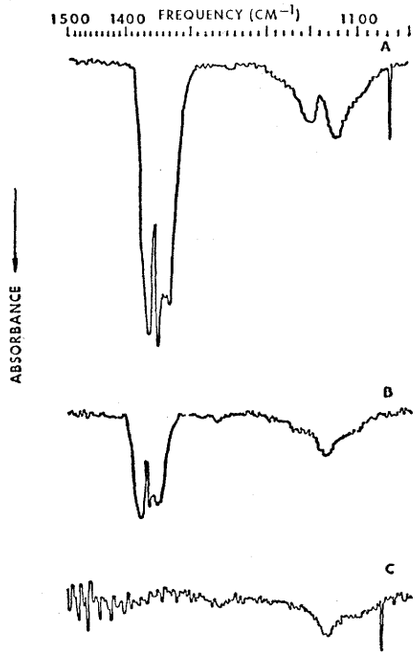

Fig. 1. (A) Infrared absorption bands of SO_2. Path length 10 cm; NaCl windows. (B) Infrared absorption of SO_2 after passage of electrical discharge. Path length 10 cm; NaCl windows. (C) Infrared absorption band of gas produced by *Desulfovibrio* collected in liquid N_2 trap. Path length 10 cm; NaCl windows.

(growth and blackening in 17 hours) and acted as a hydrogen donor for BV reduction.

Another sample of the precipitate was similarly obtained, and the gas obtained by treatment with H_2SO_4 was analyzed in the mass spectrometer. Mass peaks corresponding to H_2S^-, SO_2^-, and SO^- were found.

The H_2S had no stimulatory effect on growth and no ability as a hydrogen donor; SO_2 similarly had no effect in stimulating growth on API agar but did promote very slight reduction of BV by *Desulfovibrio* cells. "Sulfur monoxide," now established as S_2O (*4*), was prepared (*5*) by passing an electrical discharge (about 5 kv) through SO_2 (generated by adding concentrated H_2SO_4 to anhydrous reagent grade Na_2SO_3) in a straight glass tube (74 cm long; 2.5 cm inside diameter) at low pressure (2 to 3 mm) with platinum electrodes sealed at each end. The S_2O was flushed with helium into an evacuated Brewer jar with plates inoculated with API and with BV agar. After the procedure had been repeated seven or eight times, sufficient S_2O was produced so that growth, as well as reduction of BV, was visible on the API medium within 17 hours.

The infrared spectra of SO_2 before and after electrical discharge are indicated in Fig. 1. The absorption peaks for SO_2 at 1155 and 1125 cm^{-1} were replaced by a single peak at 1135 cm^{-1}. Peaks similar to those in Fig. 1B were also obtained by passing an electrical discharge through SO_2 in S vapor with the apparatus described by Jones (*6*). The gas appears to be stable in a dry glass trap, as evidenced by the persistence of the 1135 cm^{-1} peak, for at least 24 hours. This stability is in agreement with the results of Cordes and Schenk (*7*). In Fig. 1C, there is a peak at 1135 cm^{-1}, which is identical to the peak of the product of electrical discharge in SO_2. No bands for H_2S or SO_2 were found.

The infrared spectra reported by Jones (*6*) for S_2O indicate bands at 1165 and 679 cm^{-1}. Blukis and Myers (*8*), with frozen films, found that the band at 679 cm^{-1} disappeared and the band at 1165 cm^{-1} decreased in intensity and shifted to 1300 cm^{-1} when the film was heated to 280°K. The band at 1135 cm^{-1} and the one reported by Myers at 1130 cm^{-1} are probably identical and are due to a decomposition product of S_2O, the band being caused by an S-O stretch.

Schenk (*9*) and Murthy (*10*) reported that $S_2O_3^{--}$, S^{--}, and S were produced by "SO" or S_2O (because the properties previously attributed to the presence of SO in the discharge product are shown to be attributable to S_2O) when it reacted with alkaline solutions. The $Na_2S_2O_3$ (0.02 percent) incorporated in the API agar also stimulated growth and acted as an electron donor for the reduction of BV.

The organisms may be producing SO as an intermediate in the reduction of sulfate from trypticase soy broth (7 to 8 mg SO_4^{--} as the barium salt per gram of dry medium):

$$\overset{6+}{SO_4}{}^{--} \longrightarrow \overset{4+}{SO_3}{}^{--} \longrightarrow \overset{2+}{SO} \longrightarrow \overset{0}{S} \longrightarrow \overset{2-}{S}{}^{--}$$

The SO is quite unstable (decomposes within 3 seconds) and probably forms S_2O immediately, according to the following reaction: $3\ SO \rightarrow S_2O + SO_2$ (*4*). However, SO_2 was not detected in the traps during these observations.

A large number of extracellular particles, irregular in shape, were found among the cells of *Desulfovibrio* that reduced BV on agar in the presence of the gas (produced by growth of the organism on trypticase soy broth agar plates). With the technique for extraction and crystallization of elemental sulfur from organisms (*11*), in which sulfur is extracted and crystallized from pyridine, a large number of rhombic crystals were found among the cells after several hours; this suggests that the organisms might have been producing sulfur. Until the complete products of the reaction of the gas with the agar are known, it is impossible to state whether sulfur is produced by the organisms or by a direct breakdown of S_2O with water (*10*).

The gas has an odor resembling that of H_2S in contact with air. Cordes and Schenk (*12*) also observed a similar odor from their "sulfur monoxide." Murthy (*10*) reported S^{--} as a reaction product with 2*N* NaOH. The gas S_2O, or its decomposition product, is apparently produced by *Desulfovibrio* growing on agar plates; it may be an intermediate product in the reduction of sulfate.

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23 March 1967

R Factors Mediate Resistance to Mercury, Nickel, and Cobalt

Abstract. *Fifty-five clinical isolates and laboratory stocks of* Escherichia coli *and* Salmonella *were studied for resistance to each of ten metals. Eleven clinical isolates carrying R factors were resistant to mercury, and, in each case, the resistance was mediated by a previously undefined R-factor gene. The gene was phenotypically expressed within 2 to 4 minutes after entry into sensitive bacteria, but the basis for the resistance remains undefined. Fourteen strains, 12 infected with R factors, were resistant to cobalt and nickel, but these resistances were mediated by R-factor genes in only two strains; separate R-factor genes mediated the resistances to nickel and cobalt. These and other results indicate that the genetic composition of R factors is greater than that originally defined.*

The R factors are episomes of Enterobacteriaceae described originally as mediating resistance to several commonly used antibacterial drugs. Several recent findings indicate, however, that R factors may contain genes mediating other characteristics. Chemical and biological studies suggest that the DNA of a single R factor has a molecular weight of approximately 25 million daltons, an amount sufficient to code for several dozen proteins (*1*). During studies that demonstrated that the DNA of segregant R factors is reduced in quantity and altered in composition relative to the DNA of the parent episome, certain segregant R factors that had lost only four known genetic characters were found to have lost 45 percent of their DNA content (*2*); segregant R factors that had lost a significant fraction of their DNA without loss of genetic characters have also been isolated (*1*). Moreover, certain R factors have separate genes that mediate altered susceptibility to colicins, ultraviolet light, and certain experimental antibiotics and chemical inhibitors of bacteria (*3*). My data indicate that R factors may contain previously undefined genes that mediate resistance to mercury, nickel, and cobalt.

Twelve laboratory strains and 43 local clinical isolates of *Escherichia coli* and *Salmonella*, including 20 strains infected with previously defined R factors, were screened for resistance to various metals. Salts of the following metals were incorporated into enriched agar prepared with distilled water further purified by passage through a cation-exchange resin: aluminum, cadmium, chromium, cobalt, copper, lead, mercury, nickel, silver, and thallium. The ability of the strains to form colonies was tested; strains growing in three or more times the concentration of metal that inhibited most strains were defined as resistant. The techniques used for conjugation and transduction, for selection of R-factor segregants, and for the temporary alteration of cell permeability by ethylenediamine tetraacetate (EDTA) have been described (*4*).

All strains tested were equally resistant to aluminum, cadmium, chromium, copper, lead, silver, and thallium. At 1×10^{-5} mole/liter, mercury inhibited all laboratory strains and drug-sensitive clinical isolates; 11 of 20 strains carrying R factors were resistant to $1.2 \times 10^{-4}M$ mercury (Table 1). Ten of the strains were *E. coli*, and one was *Salmonella typhimurium*. Strains resistant to drugs and mercury were mated with suitable recipients, and drug resistant recombinants were selected; in each case, bacteria that acquired drug resistance mediated by the R factor also acquired resistance to mercury. Conversely, recipients selected for the acquisition of resistance to mercury also acquired resistance to drugs. When these recipients were used as R-factor donors in subsequent matings, resistance to drugs and mercury were transferred coordinately. Furthermore, resistance to mercury was transduced along with drug resistance mediated by R-factor genes but not with the several chromosomal genes tested; and bacteria selected for the spontaneous loss of drug resistance mediated by R factors also had lost resistance to mercury. These results indicated that R factors may contain a gene or genes mediating resistance to mercury.

The following findings indicated that this gene has no previously defined expression. Resistance to mercury was mediated by R factors of fi^- and fi^+ types and with varying patterns of drug resistance. Segregant clones that had lost mercury resistance but that had the pattern of drug resistance of the parent episome could be selected from cultures of *E. coli* or *S. typhimurium*.

Several factors may affect the degrees of drug resistance mediated by R factors: the medium, the episome, host strain, and, in those instances in which resistance is mediated by inactivation of a drug, the concentration of the inoculum. Resistance to tetracycline conferred by R factors is ex-

Table 1. Concentrations of mercury that inhibit certain enterobacteriaceae. Bacteria with and without R factors were tested for mercury resistance as described in the text; the minimum inhibitory concentration prevented colony formation on agar plates and growth in broth. To determine that the treatment with EDTA did alter cell permeability, I divided the treated cultures and exposed them to either mercury or actinomycin D at 10 μg/ml. Actinomycin D prevented growth of the treated cells but did not affect untreated cells.

Strain	Episome	Inoculum (No./ml)	Medium	Minimal inhibitory concentrations (mole/liter)
		No previous treatment		
E. coli B		5×10^6	Enriched	1×10^{-5}
E. coli B	E1	5×10^6	Enriched	2.5×10^{-4}
E. coli B	E1	5×10^2	Enriched	1.2×10^{-4}
E. coli B	E1	5×10^6	Minimal	3×10^{-6}
E. coli B	E7	5×10^6	Enriched	2.5×10^{-4}
S. typhimurium LT_2		5×10^6	Enriched	1×10^{-5}
S. typhimurium LT_2	E1	5×10^6	Enriched	2.5×10^{-4}
		Previous treatment with EDTA		
E. coli B		5×10^6	Enriched	1×10^{-5}
E. coli B	E1	5×10^6	Enriched	2.5×10^{-4}