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TOXICOLOGY OF HYDROGEN SULFIDE

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INTRODUCTION

Historical Background

It is almost 300 years since the first description of hydrogen sulfide (H_2S) toxicity (1). There have, however, been few reviews and only one research conference (2) on H_2S toxicity. Numerous governmental agencies concerned with occupational health or the environment have at various times prepared documents related to regulation of H_2S exposure (e.g. 3, 4).

An excellent history of the early experience with H_2S appeared in the recent conference proceedings (5). A review in 1984 (6) included a bibliography of almost 1300 references, 196 of which were cited in the text. The general opinion was that sulfide inhibited oxidative enzymes in a manner similar to

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cyanide, particularly enzymes involved with oxidative phosphorylation, but it must be concluded that additional processes are operating. Therapeutic measures have been suggested that follow the pattern used for the treatment of cyanide poisoning. However, animal experiments have not proved their validity for postexposure therapy, and clinical reports are still scarce. Recent advances have been made in the diagnosis of H_2S poisoning, teratogenicity, and neurological and respiratory effects and have opened new possibilities for therapy.

H_2S poisoning is still a problem because of widespread environmental and occupational exposure from industrial activities, e.g. paper pulp mills, heavy-water production, urban sewers, and farming, to name but a few of the more than 70 identified commercial sources. H_2S is the predominant sulfur contaminant of natural gas and ranges in concentration from <1 to >90%. At least three epidemiological studies have recently addressed the health of populations exposed to this toxic gas (7–9).

Physicochemical Properties

H_2S is a colorless gas heavier than air ($d = 1.19$) with a molecular weight of 34.08 (10). It is the sulfur analog of water. It can be oxidized by a variety of agents to form sulfur dioxide (SO_2), sulfates such as sulfuric acid, and elemental sulfur (these products also have toxicological implications). One gram of H_2S will dissolve in 242 ml of H_2O, 94.3 ml of absolute ethanol, or 48.5 ml of diethyl ether at 20°C. Because of its lipid solubility, it easily penetrates biological membranes. H_2S evaporates from aqueous solutions (vapor pressure = 18.75×10^5 Pa). An aqueous solution will dissociate (see Equation 1), yielding a hydrosulfide anion and sulfide ion; the two pKa values are 7.04 and 11.96, respectively. At a physiological pH of 7.4, approximately one-third of H_2S exists as the undissociated form and two-thirds as the hydrosulfide anion.

Some useful conversion factors for H_2S are as follows: 1% volume = 10,000 ppm, 1 mg liter^{-1} = 717 ppm (STP), and 1 ppm = 1.4 mg m^{-3}

$$H_2S \rightleftarrows H^+ + HS^- \rightleftarrows 2H^+ + S^{2-} \qquad 1.$$

Species Comparison

The effects of acute and chronic exposure to H_2S in many vertebrates and invertebrates have been investigated from three different points of view: lethality, on commercially valuable species, and mechanism of adaptation. The reader is directed to reviews on this subject (4, 6, 11, 12). Briefly, lethality data for humans, dogs, cows, goats, monkeys, mice, guinea pigs, and rats are very similar, probably because the effect of H_2S on eukaryotic cells is similar (see ref. 11 for a discussion). Table 1 shows the effects of H_2S

Table 1 Human physiologic responses to exposure to hydrogen sulfide[a]

Concentration of H_2S ppm	mg m^{-3}	Physiological responses
0.003–0.02	0.0042–0.028	Odor threshold
3–10	4–14	Obvious unpleasant odor
20–30	28–42	Strong offensive odor ("rotten eggs")
30	42	Sickening sweet odor
50	70	Conjunctival irritation
50–100	70–140	Irritation of respiratory tract
100–200	140–280	Loss of smell (olfactory fatigue)[b]
150–200	210–280	Olfactory paralysis[b]
250–500	350–700	Pulmonary edema
500	700	Anxiety, headache, ataxia, dizziness, stimulation of respiration, amnesia, unconsciousness ("knockdown")
500–1000	700–1400	Respiratory paralysis leading to death, immediate collapse, neural paralysis, cardiac arrhythmias, death

[a]Adapted from Beauchamp et al (6) and other sources (2, 4, 108).
[b]Data require reevaluation because they are based on recollection of "knockdown" victims, who are known to have memory deficits (5).

on humans. There are some anomalies in the reported findings. Guinea pigs, but not rats, were reputed to die from exposure to 100 ppm of H_2S. This may be related to the fact that guinea pigs have a more extensive nasal labyrinth, are obligate nose breathers, and did not evolve in a high-H_2S atmosphere (sewers) like rats did (see 5). In obligate nose breathers, cellular damage, exfoliation, and mucus secretion cause the nasal passages to plug up, and the animals simply cannot breathe and hence die from lack of oxygen. This effect has been seen in comparisons of nose-breathing and tracheotomized guinea pigs exposed to cigarette smoke (W. C. Hulbert, unpublished data).

Birds (canaries) are more sensitive than mammals to H_2S: 100 ppm causes 100% mortality. It is unknown whether the mechanism is similar to that in guinea pigs or whether it is due to altered metabolic or neurological function.

Extensive studies of the effects of H_2S on aquatic vertebrates have been conducted (see ref. 14 for a review), particularly channel catfish, brown trout, walleye, northern pike, blue gill, rainbow trout, and the white sucker. H_2S affects these species at all stages of development from eggs to adults, and many effects seem related to the ability of the species to express tolerance or adaptation (15). Fish reared in sublethal concentrations of H_2S exhibited growth enhanced by 50 to 200% (14, 16), owing to fungicidal and bactericidal effects of H_2S, similar to the effects of antibiotics normally used with captive fish. However, it was also noted that the fish were significantly less active (16) and showed signs of respiratory distress. Histological analysis of the gill lamellae revealed structural alterations of the gill filaments, which were

shortened and thickened, indicating chronic irritation. Unfortunately, there have been no analyses of muscle and fat content, muscle contractility or enzyme levels, or effects of exercise, factors that may be more significant than the simple enhancement of growth. H_2S becomes a major problem in fish aquaculture during harvesting (17): when anaerobic sediments of fish ponds are disturbed during netting, H_2S levels throughout the water column are elevated to the toxic range.

Sulfide is abundant in the marine environment (especially near volcanic vents), and many vertebrate and invcrtebrate species (e.g. crabs, clams, and tubeworms) have evolved strategies for dealing with its presence. These include sulfide detoxification in the body wall, binding and oxidation of sulfide by blood components and by cytosolic factors, oxidation by mitochondria, sulfide-insensitive cytochrome *c* oxidase, and ATP production from sulfide oxidation (see ref. 18 for a review).

Target Organ Systems

Most organ systems are susceptible to the effects of H_2S; therefore, this toxic gas has often been regarded as a broad-spectrum toxicant. The biological responses to H_2S are dependent on the organ system; each system exhibits a different threshold responsiveness, perhaps as a function of concentration, time, or rate of exposure (19). Tissues most susceptible to H_2S toxicity are those with exposed mucous membranes and those with high oxygen demands. The effects of prolonged exposure to low concentrations are not well documented. It has been proposed that the toxicity may be cumulative (20) or noncumulative (21) and that the effects can be completely reversible. Recovery from acute intoxication is usually rapid and complete, depending upon exposure; however, some symptoms may persist (9) and some aftereffects may be irreversible as a result of secondary effects caused by lack of oxygen due to respiratory paralysis and/or pulmonary edema (7, 21).

NERVOUS SYSTEM

Acute exposure to H_2S leads to sudden fatigue, vertigo, intense anxiety, convulsions, unconsciousness, and respiratory failure. After resuscitation, victims may suffer coordination and psychiatric disturbances, including hallucinations and amnesia. Chronic exposure leads to a variety of physiological and psychological effects (see Table 2). Early neurological studies concerned the increase in respiratory rate seen in moderate H_2S exposures; this increase was attributed to stimulation of peripheral chemoreceptors (6). However, little progress had been made in describing the neurological sequelae of high H_2S exposure until the advent of current electrophysiological techniques.

Brain Sulfide Content in Poisoning

The sulfide (S^{2-}) concentration present in tissues following poisoning was unknown until recently. A recently developed, extremely *sensitive* (2 μg liter^{-1}) method (22, 23), specific to S^{2-} and ideal for analysis of tissue, has defined the appropriate concentration of H_2S or its salts for experimental use in the brain. The method is 50-fold more sensitive than earlier methods (24). Surprisingly, both rats and humans have a relatively high endogenous level of S^{2-}: 1.57 μg g^{-1} for whole brain and 0.67 μg g^{-1} in the midbrain. It would be of interest to know the S^{2-} levels in brains of ruminants, since they produce large quantities of H_2S. Recently bovine brain levels of S^{2-} were reported to be about 5.3 μg g^{-1} (24a), although it is not known how the HPLC method used compares with the method used for rats and humans (22, 23). At the 50% lethal dose (LD_{50}) of NaHS (15 mg kg^{-1}) the level of S^{2-} in rat brain was approximately 3.1 μg g^{-1} ($\approx$75 μM). By contrast, another recent method for tissue S^{2-} analysis (25, 26) gave much lower concentrations in the brain. Significant to its effect on respiration, sulfide is selectively taken up by brain stem compared with other brain areas (27). Inhalation of 1600 ppm of H_2S and intraperitoneal (i.p.) injection of 30 mg of NaHS kg^{-1} (LD_{100} doses within 4 min) produced indistinguishable increases in S^{2-} levels in the brain (28).

Brain Neurotransmitter Content

Because of their essential role in central nervous system (CNS) function, the content and release of neurotransmitters during acute and chronic exposure to H_2S or sulfide salts have been determined. Acute i.p. treatment with NaHS ($2\times LD_{50}$) increased the concentrations of alanine, aspartate, γ-aminobutyrate (GABA), glutamate, glutamine, glycine, and taurine selectively in the brain stem; minor or no changes were seen in other brain areas (29). This dose also increased serotonin (5-HT), dopamine, epinephrine, and norepinephrine levels in the brain stem, the only region where all four amine levels changed (30). These changes in catecholamine and 5-HT levels are due to inhibition of monoamine oxidase (MAO). Acute treatment with sulfide also inhibits acetylcholinesterase (31) and Na^+/K^+ATPase (see Electrophysiological Effects). Reversal of MAO inhibition was achieved ex vivo by removal of bound sulfide with the persulfide reagent dithiothreitol (32).

Experimental handling of rats produced increases in brain stem glutamate, glutamine, and taurine levels. Subacute treatment with NaHS (0.5x LD_{50}) resulted in a reduction of this stress-induced increase (33). This dose of NaHS had no effect on amino acid levels in brain stems of mice (34). Chronic exposure depressed brain amino acid transmitters (see Reproduction and Development). Therefore, it appears that degradation of amino acids (and

amines) is inhibited by acute exposure, but that synthesis is also inhibited by chronic exposure.

Neurotransmitter Release

Release of amino acids has been studied by push–pull perfusion (35) because of evidence that NaHS depresses synaptic transmission presynaptically (36–39). Two paradigms were used: (*a*) NaHS (LD_{50}) was given i.p. or (*b*) 3–4 μg of NaHS ml^{-1} (the concentration of S^{2-} in the brain after administration of LD_{50}) was included in the perfusion medium. Perfusates from surviving animals revealed that most changes in amino acid release in the hippocampus or caudate-putamen were immediate or delayed increases (40, 41). However, in the brain stem reticular nucleus, the only change was a delayed decrease in glycine release (42). These results do not provide evidence that sulfide inhibits transmission by depressing transmitter release.

Electrophysiological Effects

Many in vitro neuronal preparations have been used as models in the study of the actions of sulfide, including those discussed below. Ideally, studies of respiratory rhythm generator cells would be desirable, but the technical difficulties of intracellular recording from a sufficient number of these neurons has led to the use of dorsal raphe as a typical midbrain nucleus. The use of the rat hippocampus may relate to the memory losses that are common in survivors of sulfide poisonings.

FROG SYMPATHETIC GANGLION In view of the changes in catecholamine levels and in acetylcholinesterase activity (31) after administration of sulfide, effects of NaHS in the frog sympathetic ganglion have been investigated by the sucrose gap method (43, 44). With this technique, α_2-epinephrine, muscarinic and nicotinic receptors, and Na^+/K^+ATPase electrogenic pump activity can be studied (45, 46). NaHS reversibly depolarized the ganglion, but did not alter the depolarizing effect of nicotine. However, the hyperpolarizing effects of epinephrine and muscarine were both reversibly increased by NaHS. The hyperpolarizing response to pump activation did not change while NaHS was present, but was greatly potentiated after removal of the NaHS, recovering to normal after 45 min. A similar effect occurred in mammalian neurons (see Hippocampal CA1 Neurons, below). It is remarkable that these neurons were exposed to sulfide for extended periods without being irreversibly damaged.

It is tempting to suggest that the sulfide-induced depolarization is due to inhibition of the electrogenic pump, either directly or from inhibition of ATP production; however, this seems unlikely since Na^+/K^+ATPAse activity in the presence of sulfide was equal to that in the control.

CRAYFISH SENSORY NEURON This preparation *(Procambarus clarkii)* was used to study the effect of sulfide on action potential (AP) generation and conduction, by using extracellular recording (47, 48). Sulfide salts ($<10^{-4}$ M) caused an initial brief ($\approx$1 min) block of APs, then a prolonged enhancement of AP amplitude, and then another brief inhibition of APs upon washout. Higher concentrations of sulfide caused irreversible changes. In the absence of intracellular studies, the reason for these changes remains to be elucidated. Sulfide did not, however, alter the rate of AP conduction. This is similar to earlier results obtained with frog sciatic nerve, for which large concentrations (1–100 mM) of sulfide only slightly reduced the conduction velocity (49).

MOUSE NEUROBLASTOMA CELLS Murine neuroblastoma cells, clone N1E-115 derived from sympathetic ganglia, were used to study tetrodotoxin (TTX)-sensitive Na^+ channels by the patch–clamp method (50). Ca^{2+} and K^+ currents were blocked by Cd^+, Cs^+ and tetraethylammonium (TEA^+). NaHS, even as high as 10 mM, completely failed to alter the TTX-sensitive Na^+ channels. As controls for sulfur-containing compounds, trials were also done with taurine and cysteic acid, neither of which alone affected the Na^+ channels. It was discovered, however, that the combination of NaHS and either amino acid completely and reversibly inhibited the channels. Other sulfur-containing reagents (0.8 mM β-mercaptoethanol and 2 mM dithiothreitol) inhibited Na^+ channels by themselves. In in vitro situations it seems unlikely that sulfide will affect APs. However, in vivo, where free taurine levels are normally high and further increased by sulfide, taurine could play a role in H_2S depression of CNS function.

HIPPOCAMPAL CA1 NEURONS CA1 neurons in hippocampal slices have been studied in current clamp by using potassium acetate–containing intracellular electrodes (36–38; R. J. Baldelli, R. J. Reiffenstein & W. F. Colmers, unpublished data). Slices were treated with 27–200 μM NaHS. The amplitude, duration, and threshold voltage of APs in CA1 neurons were unaffected by 80 μM NaHS. The initial response of these neurons to NaHS was a rapid, reversible, concentration-dependent hyperpolarization (IH) and reduction of input resistance. Both effects were maximal at 160 μM NaHS. The reversal potential for the conductance change was -100 mV, or slightly less than the calculated E_K for these cells. An even more striking effect of NaHS was a further hyperpolarization (WOH) that occurred immediately after washout of the NaHS. This also was concentration dependent, being maximal at >200 μM. Synaptic transmission, measured by extracellularly recorded

population spike and EPSP field potentials, and by intracellular EPSPs, was depressed by NaHS.

Pharmacological investigation of the IH and the WOH suggests that these are due to the opening of a K^+ channel and to activation of Na^+/K^+ ATPase, respectively. Maximal responses to 200 μM NaHS were studied (36–38; R. J. Baldelli, R. J. Reiffenstein & W. F. Colmers, unpublished data). Extracellular application of 1 μM TTX (to block evoked transmitter release), 1 mM 4-aminopyridine (4-AP) to block the somatic "A" and "D" K^+ currents, or 30 μM muscarine to block the voltage-dependent "M" current, did not alter the IH. However, it was reduced by 50 mM (but not 10 mM) TEA^+ (36–38) and by 2 mM Cs^+. Extracellular Ba^{2+} (1 mM), extracellular Ba^{2+} plus Cs^+, and intracellular Cs^+ blocked the IH and unmasked a depolarization response to NaHS. Conductance changes were significantly reduced only by Ba^{2+} or intracellular Cs^+ (36; R. J. Baldelli, R. J. Reiffenstein & W. F. Colmers, unpublished data). Intracellular release of neither MgATP nor Cl^- reduced the IH and change in conductance.

Thus, it seems reasonable that the IH is due to increased conductance of a K^+ channel. Comparison of the data with two recent compendia of K^+ channels and their inhibitors (52, 53) makes it relatively easy to determine what that channel is not. It does not involve the fast transient voltage-dependent I_A, nor I_D, nor the Ca^{2+}-activated nonspecific cation channel (I_t), since 4-AP was ineffective, nor I_M, as muscarine did not inhibit it but Cs^+ did. Because sulfide blocks oxidative phosphorylation (6), the consequent depletion of ATP could activate ATP-sensitive K^+ conductances, but injection of ATP did not alter the IH. The conclusion that a gK_{ATP} is not involved must be tempered as the mechanism of K^+-channel control by ATP is not understood; this action of ATP may also be inhibited, given the number of enzymes known or inferred to be inhibited by sulfide. TEA^+ blocks many K^+ currents, including voltage-dependent "delayed rectifiers" and some Ca^{2+}-activated K^+ channels (52). The cardiac-type inward rectifier (I_{IR}) fits the antagonism data (inhibition by TEA^+, Cs^+, and Ba^{2+}, but not by 4-AP).

None of the procedures listed above, except intracellular Cs^+, inhibit the WOH (36–38; R. J. Baldelli, R. J. Reiffenstein & W. F. Colmers, unpublished data). Moreover, the NaHS-induced K^+ conductance changes often recovered before the maximum WOH was reached. The Na^+/K^+ATPase inhibitor strophanthidin (3–30 μM) did inhibit the WOH; however, this treatment depolarized the neurons, and NaHS caused further depolarization. Thus it is likely that the WOH results from activation of Na^+/K^+ATPase, just as in the frog sympathetic ganglion. The NaHS-induced depolarization seen in the presence of strophanthidin suggests that sulfide does inhibit Na^+/K^+ATPase in this preparation, although this is not so clear when using frog ganglia.

Neither did the treatments listed above affect the depression of synaptic transmission (36–38), which suggests a presynaptic effect. However, this is not consistent with the release experiments, evidence that depolarization by iontophoresed glutamate pulses can be inhibited (36), or inhibition of glutamate binding to hippocampal neuronal membranes (K. Fung, M. W. Warenycia, S. B. Kombian & R. J. Reiffenstein, unpublished data), all of which suggest a postsynaptic effect.

DORSAL RAPHE NEURONS Effects of NaHS similar to those obtained in hippocampal CA1 cells have also been observed in voltage-clamped serotonergic (55) dorsal raphe neurons (39). Some cells responded to NaHS with an IH (outward current) followed by a WOH, as in CA1 neurons. However, in some of these raphe neurons the IH was superimposed on a sulfide-induced depolarization (inward current). In both cases, blockade of the IH with Ba^{2+} plus Cs^{+} revealed an underlying inward current. Some neurons responded only with initial inward currents, followed by the same WOH. In all cases the WOH was blocked by strophanthidin. The NaHS-induced inward currents were also occluded by the strophanthidin, showing that the depolarization was due to at least partial inhibition of Na^{+}/K^{+}ATPase by sulfide. A few neurons appeared to be unresponsive to sulfide.

Although the mechanism of action of H_2S on neurons is not completely clear, sulfide can activate K^{+} conductances in at least two very different kinds of neurons, and these conductances are sensitive to extracellular application of Ba^{2+} and Cs^{+}. Upon blockade of these K^{+} currents, all neurons showed a depolarizing response to NaHS, which is caused by a voltage-independent (at least in dorsal raphe neurons) inward current and which may well be due to suppression of outward current generated by the Na^{+}-K^{+} exchanger. Dorsal raphe neurons are variably affected by sulfide, perhaps reflecting the relative contributions of the Na^{+}/K^{+}ATPase activity and the Cs^{+}-Ba^{2+}-sensitive K^{+} conductances in their resting state. All cells that responded to NaHS also showed the outward current response to washout of NaHS. This action of sulfide on Na^{+}/K^{+}ATPase in mammalian CNS neurons is rapidly and completely reversible. Clearly, other types of neurons will have to be sampled before a generalization about neural mechanisms of sulfide toxicity can be made. At present, the relatively uniform responses of hippocampal CA1 neurons do suggest that inhibition may be the reason for temporary memory deficits which occur in high H_sS exposure. The WOH, and potentiation of other inhibitory mechanisms, may well slow the return of function.

HYPOXIA AND ANOXIA Are the effects of H_2S on nervous tissue simply those due to inhibition of oxidative metabolism? Similar membrane potential responses to those induced by sulfide have been shown in vitro during anoxic

or hypoxic conditions (56–60), but there seem to be pharmacological dissimilarities. The anoxia-induced IH in hippocampal pyramidal neurons was reported to be blocked by 4-AP (0.4 mM) (56). Another group (57) found that Ba^{2+} (0.5 mM) blocked the IH but that 4-AP (0.2–1.5 mM) and Cs^{+} (2–4 mM) were ineffective (57, 58). Neither group found TEA^{+} (3–10 mM) effective; however, less than 50 mM TEA^{+} was ineffective against NaHS (36). It has been variously concluded that the anoxia-induced IH is in part due to a Ca^{2+}-sensitive gK (59) and also due to a muscarine- or carbachol-sensitive gK (I_M) (60). None of these anoxia data are consistent with the sulfide pharmacology. A reoxygenation hyperpolarization similar to the sulfide WOH has also been observed (57, 58). This also seems to be due to the electrogenic pump, since it was abolished by low K^{+} concentrations or 1 μM ouabain. The action of sulfide is often compared to that of cyanide (6), which does block oxidative metabolism; however, on the basis of comparison of the actions of cyanide and sulfide on frog nerve (49), it has been concluded that sulfide acts by other than metabolic actions. At this time it seems that the processes caused by sulfide and simple anoxia, although producing a similar end point, are not completely identical. In view of the controversy over the actions of cyanide and sulfide and the role of chemically-induced "anoxia," it would appear essential to test the actions of cyanide in a similar manner.

RESPIRATORY SYSTEM

The effects of toxic gas exposure on the respiratory system have been the focus of intensive investigations (see ref. 61 for a review). One of the hallmarks has been the change in bronchial reactivity to inhaled nonspecific agonists. Although the patterns vary, at some level of exposure there is an increase in bronchial reactivity or hypersensitivity and the expression of asthmalike responses. However, there are few animal studies and even fewer human studies that have examined this very important effect on the pulmonary system.

Clinical Manifestations

In reviewing the case studies of accidental exposure, the respiratory complaints are the second major group of symptoms reported after neurological ones. The most prevalent respiratory symptom following accidental exposure to H_2S is dyspnea (7). In fact, dyspnea accounted for symptom complaints in 23% of 250 H_2S-exposed workers who filed claims with the Workers' Health and Safety Compensation Board in Alberta, Canada. Other prevalent symptomatic complaints in that study were sore throats, coughs, and chest pain. In nine of these workers given pulmonary function tests, three showed an obstructive pattern. Other respiratory signs and symptoms seen less frequently

were pulmonary edema, cyanosis, and hemoptysis. One of the complications following exposure to H_2S is the development of pneumonia, which may be related to the inhibitory effect of H_2S on alveolar macrophages and their subsequent ability to inactivate bacteria (62).

Only one study has evaluated the effects of environmental levels of H_2S on pulmonary function (8). This was an examination of the effects of living downwind from a natural-gas refinery. Although the investigations did not assess hypersensitivity by challenge with either H_2S or a nonspecific agonist such as methacholine, they did show that there was an excess of respiratory symptoms in the exposed area, especially in children from 5 to 13 years of age and in never-smokers over 14 years of age. Unfortunately, their study did not assess the direct effects of H_2S, but, rather, those of the combined emissions from the gas refinery.

A recent study (63) evaluated the effects of inhaling H_2S, 2 ppm for 30 min, on pulmonary function in a cohort of pulp mill workers who either were asymptomatic for asthma or had symptoms of asthma. No effect on airway resistance or specific airway conductance in the asymptomatic workers was found. In the asthmatic subjects there were nonsignificant increases in airways resistance (26.3%) and decreases in specific airway conductance (8.4%). The investigators concluded that exposure for a relatively short time to H_2S concentrations appreciably higher than those existing in ambient air in the pulp mill does not cause noticeable effects on respiratory function; however, in 2 of the 10 asthmatic subjects, changes greater than 30% in both resistance and conductance were found, indicating airflow obstruction.

These results must be evaluated in light of two factors. First, no non–pulp mill workers were included in the study. Second, as it is known that a self selection takes place in workers in the pulp and paper industry, such that only those who can tolerate the fumes work in the environment (64), this may significantly pre-bias any results.

It is significant that symptoms of obstructive air flow only occurred in the asthmatics. One might speculate that in a more normal population, there may be more responses to the inhalation of H_2S in asthmatic subjects, consistent with their hypersensitivity to toxic gases. To date, there have been no pulmonary function studies evaluating a cross-section of the general population to exposure to H_2S. Until that is done, the effects of H_2S on pulmonary function and bronchial reactivity in humans will remain speculative.

Animal Studies

There have been two major animal studies that have examined the acute and subchronic effects of inhaling H_2S on pulmonary function, bronchial reactivity, and lung histology (65–70). The subchronic experiments were designed to

assess the applicability of the current occupational standards for exposure to H_2S in the workplace. Currently proposed regulations (71) for exposure of workers to H_2S are a time-weighted average-threshold limit value (TWA-TLV®) of 10 ppm for a week consisting of five 8-hr days and a time-weighted average short-term exposure limit (TWA-STEL®) of 15 ppm for 15 min. These levels were initially intended to prevent eye injuries (11), but the standards were never tested in animals to determine whether they protected against pulmonary injury.

SUBCHRONIC LOW-LEVEL INHALATION Subchronic studies (65, 66, 68–70) showed that the exposure to H_2S at 1, 10 and 100 ppm for 8 hr per day for 5 weeks had no effect on baseline measurements of airways resistance (R_L, a measure of central airway function), dynamic compliance (C_{dyn}, a measure of peripheral airway function), tidal volume, minute volume, or heart rate. It was also found that the maximal changes in R_L and C_{dyn} with an aerosol methacholine (MCh) challenge were comparable for all groups. This was unexpected in view of reported damage to the nasal mucosal epithelium following H_2S exposure (72). It was anticipated that baseline measurements would be elevated, reflecting changes in airway caliber due to injury.

Although baseline and maximal responses to MCh were unchanged, there was a leftward shift in the R_L and C_{dyn} dose-response curves (DRC) for some rats: these responded maximally to a 10-fold-lower MCh dose at all H_2S exposure levels. Sensitivity (concentration of agonist causing a half-maximal response) and reactivity (rate of the response) varied widely; however, cluster analysis did show distinct groupings of response, with clear separation between animals responding like the controls and hyperreactive individuals (70).

Histologic examination of the trachea and lungs in normal and hyperreactive rats revealed only subtle differences in structure; neither an inflammatory infiltrate nor increased numbers of mast cells were seen in the hyperreactive animals. In many rats exposed to H_2S, there were two consistent dose-related peculiarities: proliferation of the ciliated cells in the tracheal and bronchiolar epithelium, and a lymphocyte infiltrate of the bronchial submucosa in addition to the recognized bronchoalveolar lymphatic tissues. Neither change has been reported to be causally related to bronchial reactivity.

The mechanism of the hyperreactivity may be related to increased airway mucosal permeability. Low concentrations of thiols such as H_2S and CH_3SH are known to markedly increase permeation of macromolecules across the porcine oral mucosa (73). A series of studies on the acute effects of cigarette smoke inhalation have shown that increased bronchial reactivity is associated with increased mucosal permeability (74).

The identification of individual rats that were hypersensitive after inhaling

H_2S has significance for accidental human exposure to H_2S and possibly to other toxic gases. In some rats the hyperreactive response occurred after they had inhaled only 1 ppm, which is only twice the concentration recorded in the city of Edmonton, Canada, during the 1982 gas well blowout 138 km distant. Whether prolonging the exposure would recruit more individuals into the hyperreactive subgroup is not known, but the DRC seems to imply this. These findings indicate that the occupational exposure standards that were established upon other criteria (eye damage) may not necessarily protect the pulmonary system from damage.

ACUTE HIGH-LEVEL INHALATION The acute effects on pulmonary function and bronchial reactivity of inhaling moderately high concentrations of H_2S for 1 h were investigated by using guinea pigs (67, 68). There were no effects on baseline R_L or C_{dyn} for the control, 100-ppm, and 500-ppm groups, but there was a leftward shift in the aerosol MCh DRC that was related to the H_2S dose. Sensitivity and reactivity increased 10- and 3-fold, respectively, for R_L and 3- and 4-fold, respectively, for C_{dyn}. Thus, the central and peripheral airways of the animals were more sensitive to inhaled MCh, and the slope of the DRC was steeper after exposure to H_2S. There are several possible explanations. Because baseline measurements were not significantly different, it is doubtful that the starting airway caliber is involved. Increased mucosal permeability is possible, and its relationship with hyperreactivity is well documented, as discussed above. Another possible mechanism is a change in the smooth muscle itself. Alterations in the sensitivity of the irritant receptors are also possible although not likely, given the lack of effects of H_2S on APs and nerve conduction (see Electrophysiological Effects).

The hypothesis that the increased sensitivity and reactivity were due to increased mucosal permeability has been tested (75). First, following exposure to 100 and 500 ppm of H_2S, the mucosa became hyperpermeable to dextran (FITC-T-40; molecular weight, 40,000) in a dose-related way. Transmission electron-microscopic analysis showed precipitation of the dextran in the intercellular spaces in airway tissues from the animals that had been exposed to H_2S but not the controls, corroborating the physiological measurements on dextran in blood. Second, when MCh was injected intravenously (i.v.) (bypassing the effect of the airway mucosa by delivering the MCh directly to the smooth muscle), it was found that there was no difference in the DRCs between any of the control or H_2S-exposed animals. This confirmed that bronchial hyperactivity following the acute exposure to H_2S involved hyperpermeability of the airway mucosa, which facilitates the access by inhaled agonists to the underlying smooth muscle. The mechanism of separation of the tight junctions responsible for the hyperpermeability remains speculative, but is believed to involve alterations in the interaction of the

cytoskeleton at the plasma membrane, influencing the integral components of the tight junction, or focal changes in intracellular and/or intercellular levels of Ca^{2+}, or both.

Other pulmonary studies have shown that rats exposed to H_2S at 10, 200, or 400 ppm for 4 hr develop necrosis of the nasal epithelium followed by exfoliation of ciliated and olfactory mucosal cells but not of the squamous epithelial cells; these cytotoxic effects, and polymorphonuclear exudation and pulmonary edema, were dose related (76). Resolution proceeded much faster in nasal than in olfactory mucosa, as olfactor cells were still exfoliating 44 hr after exposure. This may relate to clinical reports of olfactory fatigue or paralysis.

The per-acute effects of inhaled H_2S and injected NaHS (LD_{100}s) on the lungs of rats were studied (77). At necropsy, all rats in the H_2S group had gross and histological pulmonary edema, characterized by massive extravasation of eosinophilic fluid into the bronchoalveolar space. By contrast, the NaHS group were unaffected. Although the levels of S^{2-} achieved in the brain were identical (28), the effects on the lungs were not. It is not known whether this was due to a concentration difference at the site of action or whether direct access to the alveoli is required.

The DRC for rats exposed to H_2S is very steep, indicating the possible existence of a sensitive physiologic threshold that, once breached, leads to pulmonary edema and, shortly thereafter, to death (78). Others have shown that when rats are pretreated with capsaicin, the C-fibers that innervate the central airways are depleted of substance P, thus removing the airway defense properties mediated by tachykinins (79). When these capsaicin-treated animals were exposed to a concentration of H_2S that causes 20% mortality in controls, they all died. As well, the capsaicin-pretreated animals died faster than controls and showed more pronounced pulmonary edema.

REPRODUCTION AND DEVELOPMENT

Before 1984, the effects of H_2S on reproductive processes were not well established. The few reports in the literature originated from studies of the effects of "thermal" mineral waters (containing sulfides). These studies claimed that H_2S may be teratogenic and embryotoxic, as well as suppressing the spermatogenic index. The studies lacked scientific merit because they provide few, if any, details of methodology, results, statistical analysis, or adequate controls (6). Adverse effects on reproduction following chronic exposure to H_2S have been described (80); however, the gas was coadministered with CS_2, which is well known to be associated with increased toxicity of reproduction (81).

Reproduction

These ambiguous effects and uncertainty of the results warranted further research on H_2S-induced effects on reproduction and development. Recent well-controlled studies have provided definitive proof that chronic low-dose (<100 ppm) exposures to H_2S do not produce any significant adverse effects on reproduction in rats (82, 83). At parturition, however, it was observed that some dams exhibited a dose-dependent increase in delivery time (dystocia) that could have resulted in loss of fetuses owing to asphyxiation (82). In vitro studies provided support that this effect may be a result of a reduction or blockade of oxytocin receptors (85).

Development

Pups exposed to H_2S (<75 ppm) in utero and neonatally to day 21 postpartum developed normally, with only a subtle decrease in time of ear detachment and hair growth (82). No significant differences were seen in growth and weight gain, although depression of weight gain occurred in adult rats. Measured glucose levels were significantly elevated in maternal blood, and serum triglycerides were decreased in pups and dams; however, there was no evidence of alterations in alkaline phosphatasc, lactatc dehydrogenase, or serum glutamate transaminase (83).

The developing or immature organism lacks many defensive mechanisms such as metabolic processes (86). An incomplete blood–brain barrier and the rapid growth characteristics make the brain particularly susceptible to various toxicants (86). Until recently, there was no conclusive evidence that H_2S altered the developing brain. An isolated report (87) suggested that the retarded development and listlessness of breast-fed infants of mothers working in rayon factories was due to H_2S. A brief case report (88) described a 20-month-old child with encephalopathies that may have been due to chronic H_2S exposure and that reversed spontaneously.

We have recently documented that chronic exposures to low levels of H_2S (20–75 ppm) can produce subtle but significant alterations in the architecture and growth characteristics of the developing brain in rats (89). Rat fetuses (in utero) and neonatal pups were chronically exposed to low concentrations of H_2S (50–75 ppm, 7 hr per day, 7 days per week) from 5 days postconception to 21 days postpartum. The growth patterns of the dendritic fields of developing cerebellar Purkinje cells were evaluated by using a digitizing method of analysis (89), which quantitates growth of the dendritic trees. Exposure to 20 and 50 ppm of H_2S produced longer branches, an increase in the vertex path length, and variations in the number of branches in particular areas of the dendritic field. The cells also exhibited a nonsymmetrical growth pattern at a time when random terminal branching is normally occurring (89). In another

study, changes in the amino acid content of developing rat brain tissue were determined (90). On postnatal day 21, aspartate, glutamate, and GABA levels in the cerebrum and aspartate and GABA levels in the cerebellum were significantly depressed. At this critical time of development, it is possible that a decrease in neurotransmitter content may reflect a cellular loss or an alteration in the synthesis or release. In preliminary studies we observed that the mean number of cerebellar Purkinje cells was increased by approximately 20% (48), suggesting that the decrease in GABA was unrelated to loss of Purkinje cells. It also appears that the normal perinatal loss of Purkinje cells is suppressed by H_2S. Exposure to low concentrations of H_2S also resulted in an initial increase in the level of taurine in the pups, which may have resulted from maternal sources (91). The return to control levels coincided with the approximate time of establishment of the blood–brain barrier (91), but may be a result of development of capacity to metabolize taurine (90). In addition, the dams exhibited inhibition of alkaline phosphatase and cytochrome oxidase in brain (90). These studies provide evidence that chronic exposure to low concentrations of H_2S does affect development of the CNS and may contribute to possible long-term abnormalities in motor function and behavior. It is not yet established whether these effects are reversible in the continued presence of H_2S or following removal; a study longer than 21 days postpartum (i.e. at least 90 days) is required.

SECONDARY TARGET SYSTEMS

Comparatively little attention has been focused on other organ systems over the past 10 years. Below are summaries of the known effects of H_2S on the various organ systems; the reader is referred to previous reviews (4, 6, 21), or to one of the many regulatory documents, for most bibliography prior to 1982. Recent developments are specifically noted. The broad spectrum of actions and physicochemical properties of H_2S predict that all or at least most organ systems will be affected to some degree.

Eye

In both humans and animals the moist mucous membranes of the eye can be directly exposed to H_2S, and at sublethal concentrations the cornea and conjunctiva are usually irritated (keratoconjunctivitis) (6). This was the first reported toxocity of H_2S (1). Common complaints following exposure to H_2S are listed at the beginning of Table 2. If exposure continues, epithelial cells swell and blister and progress to form vacuoles, which may burst into painful but reversible ulcers on the corneal surface. This condition is often referred to as "sore eye" or "gas eye" (93). In severe cases, ulceration of the cornea has led to scar formation and permanent visual impairment. Recently it has been

shown that an increase in epithelial cells collected by an eye wash (conjunctival cells increasing relative to corneal cells) constitutes an objective measure of ocular irritation by H_2S (93).

Olfactory System

One of the most common complaints of individuals exposed to low concentrations of H_2S is the unpleasant odor. The odor threshold of H_2S is very low compared with many other chemicals (21); however, at concentrations over 100 ppm there is an apparent loss of the smell sensation, which is said to be due to olfactory fatigue. However, fatigue or paralysis of the olfactory nerve has seldom been referenced to an original source, and this well-accepted belief should be reexamined (5). Prolonged exposure may result in a lower threshold for olfactory fatigue (3), although this also has been disputed (5). Nevertheless, the odor of H_2S at low doses may not provide an adequate warning. Since sensitivity appears to diminish with age, it is likely that older persons in the workforce will have higher thresholds (21).

Skin

Although the skin is the largest organ of the body and, to various degrees, will come into direct contact with H_2S, there have been few reports on the dermal effects of H_2S. Some earlier observations at high concentrations have documented discoloration, spots, and rash. Most anecdotal reports describe reversible skin irritation or allergies that are believed to be a result of exposure to low levels of H_2S ($<$20 ppm), and a few have also documented observations at very high concentrations.

Cardiovascular System

Several clinical reports demonstrate the sensitivity of the human cardiovascular system to H_2S. Acute exposure to high concentrations has resulted in transient changes in electrocardiograms as well as decreases in blood pressure. At low concentrations, H_2S may not present any risk to the cardiovascular system (94). Since cardiac muscle is one of the tissues with a high oxygen demand (95), it is likely that some effects of H_2S may not be directly on the myocardium, but secondary to anoxia due to pulmonary edema and CNS effects. Clinical observations have been well substantiated by animal studies. It was demonstrated that acute or chronic exposure of rabbits and guinea pigs to 72 ppm of H_2S or intravenous Na_2S produced ventricular extrasystoles (6). NaHS caused arrhythmias and a progressive increase in tension in isolated rat atria (R. J. Reiffenstein & B. Phipps, unpublished data). Histochemical examination of myocardial tissue from H_2S-exposed rabbits also revealed enzymatic changes, suggesting a possible interference with oxidative metabolism and therefore alteration of ionic conductances in the excitable

tissue. In other studies, chronic exposure to lower concentrations of H_2S (10–80 ppm) showed no effect on the heart rate or blood pressure of mice and rats (97). There still exists the possibility that individuals with some form of cardiovascular disease may be more sensitive to the effects of H_2S and thus form a part of society at greater risk of H_2S toxicity (9, 98).

Hepatic Tissue

The few reports describing possible effects of H_2S on liver function are conflicting and inadequate (6). Clinical studies have suggested that the incidence of cholecystitis, cholangitis, and cholelithiasis was higher than normal in oil refinery workers. Data from animal studies are less helpful, describing no effects in mice or rats chronically exposed to H_2S up to 80 ppm (97) or conversely, decreased bile flow in rats treated with 40 mg of Na_2S kg^{-1} (99), enlarged paled livers of mice exposed to 63 ppm for 16 hr, and severe hyperemia of monkey liver exposed to 500 ppm (6).

Renal System

At low concentrations, H_2S appears to have little effect on kidney morphology (97) or enzyme activity; however, earlier studies described changes in the color of mouse kidneys exposed to 63 ppm for 16 hr and pathological changes in exposed rat kidney (6). Histochemical studies of H_2S-exposed rabbits revealed some reductions in levels of renal enzymes such as succinic dehydrogenase and alkaline phosphatase, as well as enhancement of acid phosphatase (6). These results, similar to those described for liver function, are inconclusive and require more definitive and controlled studies with both acute and chronic paradigms.

Gastrointestinal System

It is common for individuals exposed to H_2S to experience gastrointestinal symptoms such as nausea, vomiting, diarrhea, and pain. The effects appear to be reversible and non-life-threatening in both humans and other animals (97).

Hematopoietic System

The few documented studies of the effects of H_2S on the hematopoietic system have reported variable results. Both increased and decreased erythrocyte counts were recorded in animals exposed to 1–50 ppm and to very high (>900 ppm) levels of H_2S. An increase in a variety of hematological parameters, but a decrease in erythrocyte numbers in mice exposed to H_2S have been reported (100). In contrast, other studies (97) observed no changes in the hematological parameters following chronic exposure to H_2S at 10–80 ppm. A decrease in enzymatic activities associated with heme synthesis occurs in humans exposed to H_2S plus methylmercaptan during wood pulp production

(101, 102). Results of interaction of H_2S with hemoglobin to produce sulfhemoglobin are also contradictory (5, 6).

Immune System

There are a few studies which suggest that H_2S interferes with the immune system. It was concluded in one study involving *Staphylococcus* challenge in rats exposed to 45 ppm of H_2S that secondary infection due to depression of macrophage function may occur following H_2S exposure (62). This was recently confirmed in an epidemiology study (8).

The possibility of allergic or enhanced anaphylactic response has also been implicated in one early study (6), but the response in rabbits was opposite to that in guinea pigs; this creates some doubt about the validity of the results and their interpretation.

Endocrine System

Possible alteration of endocrine functions has also been suggested by a decrease in milk production in cows exposed to 20–50 ppm of H_2S and by a 50% increase in plasma cortisol levels in goats at 100 ppm. Dose-dependent lesions of rat thyroid gland following administration of 14–28 ppm of H_2S have also been reported (6).

Psychological Effects

Behavioral and psychological effects of H_2S (see Table 2) have been discussed in several earlier studies, and there is a recent report of persistent cognitive impairment of three patients following acute exposure to H_2S (103). A recent case of "knockdown" (unconsciousness) resulted in permanent retrograde amnesia (I. M. O. Vicas, personal communication). The offensive odor is often interpreted as dangerous or life-threatening, and this could induce a variety of both psychological and neurophysiological reactions.

Carcinogenesis

There has been very little activity in research concerning the carcinogenic, mutagenic, or teratogenic effects of H_2S in humans and other animals; the reported genotoxic effects may be limited to cytotoxicity (6). Further investigations in validated test systems are obviously required.

CLINICAL APPLICATIONS

Diagnosis of exposure to H_2S is usually a matter of circumstance. There are a wide variety of symptoms (Table 2), not all of which may be present.

Table 2 Clinical symptoms after H_2S exposure[a]

"Felt ill"	Headache	Depression
Visual "fogging"	Insomnia	Irritability
Conjunctivitis	Sore throat/cough	Amnesia
Photophobia	Chest pain	Disequilibrium
Tearing	Dyspnea	Convulsions
Eye pain	Hemoptysis	Pulmonary edema
Nausea	Lethargy	Cyanosis
Vomiting	Abnormal peripheral reflexes	Unconsciousness
Anorexia	Weakness of extremities	Bradycardia

[a] Compiled from several sources (2, 7, 108).

Forensic Detection of H_2S Poisoning

Confirmation of sublethal exposure has been difficult until the recent development of a simple method for detecting blood sulfide levels (105). Even so, the procedure is too long to provide rapid diagnosis. Sulfide levels are still elevated in blood samples taken 2 hr after exposure (101, 102). In addition, some enzyme levels remain depressed long after the exposure (101, 102). After lethal exposures, the brain sulfide content can now be measured for forensic purposes (22, 23). To date this procedure has been used to determine the cause of death in four cases of suspected H_2S poisoning (106); one of these was determined not to directly involve H_2S. The use of dithiothreitol in this test should improve the differentiation between normal and poisoned individuals, although this has only been tried in animals thus far (32).

Therapeutic Management of H_2S Poisoning

The initial events in recovery are the most important, and immediate removal to fresh air is paramount (but inadequately equipped rescuers often become victims). Most victims, even though they may be unconscious, appear to recover spontaneously if they are breathing. If they are not, assisted breathing should be instituted immediately, with full cardiopulmonary resuscitation if there is no heartbeat (107). Given the low level of H_2S exhaled, there would appear to be no danger to the rescuer in the use of mouth-to-mouth respiration (107). Only then should other measures be instituted. Two such approaches have been advocated: scavenging sulfide with methemoglobin formed by administration of nitrites, and administration of hyperbaric oxygen.

NITRITES Nitrite does protect against subsequent poisoning in animals (108), but there are few cases in which treatment after exposure can be shown to have affected the outcome. One early survey (109) suggested that nitrites were of little use. Four recent case reports (110–113) give conflicting evi-

dence of the value of nitrites. It has been suggested (5) that nitrites may be of use only if given within minutes after the H_2S exposure.

OXYGEN Although nitrite did protect against sulfide toxicity, the same study showed that 100% O_2 at atmospheric pressure had no beneficial effect (108). There are two case reports of the use of hyperbaric O_2 therapy (112, 113) (in which nitrites were also used). In the first there was evidence of pulmonary edema, when the hyperbaric O_2 undoubtedly increased O_2 delivery. In the latter case, after extensive but ineffective treatment with nitrites, 12 treatments with hyperbaric oxygen were given over 6 days before the patient was asymptomatic. This cannot be distinguished from normal recovery. One other patient (I. M. O. Vicas, personal communication) was unconscious for several hours and regained consciousness during hyperbaric oxygen therapy; however, this individual had severe, apparently permanent, retrograde amnesia. Although this therapy is modeled after treatment for cyanide poisoning (113), it is still unclear whether hyperbaric oxygen affects the outcome of H_2S poisoning (107). Even if the increased partial pressure of the oxygen competitively reactivated oxidative cytochromes, as has been suggested (113), it is possible that the action of ATP thus produced is still inhibited by sulfide.

PERSULFIDE REAGENTS Some enzymes remain inhibited far beyond the time to apparent recovery, e.g. some blood enzymes returned to normal only 2 months after the H_2S poisoning (101, 102). This suggests that some of the sulfide remains firmly bound and is not removed by lowering the plasma sulfide levels by scavenging or metabolism. It should be possible to actively remove this HS^- ion by persulfide reagents. Dithiothreitol given 20 min before NaHS provided significant protection in rats (32, 44); however, death was too rapid for this agent to provide resuscitation if given after the NaHS. Dithiothreitol administration increases the amount of sulfide recovered from the brain tissue of poisoned animals and reverses the inhibition of MAO by HS^- (32). It also restores contractile function in oxidant-injured cardiac muscle (114). This might be doubly useful, since the 1975 report of increased cardiovascular mortality among workers exposed to H_2S (98) has recently been confirmed (9), and there are a number of reports of prolonged arrhythmias following exposure of animals to H_2S (6; see also Cardiovascular System). Interventions that actively remove sulfide from the sites causing inhibition of enzymes, or altered control of ion channels, should hasten recovery. Antidotes suitable for use in the field, especially in situations in which almost immediate death occurs, are unlikely to be found (5). However, in cases of extended unconsciousness, when the victims are hospitalized while still alive, this approach should be considered.

SUMMARY

Significant progress had been made in determining the action of sulfide on the primary target organs. It is reasonably clear that sulfide causes both K^+-channel-mediated hyperpolarization of neurons and potentiation of other inhibitory mechanisms. It is not clear whether these processes are similar to those that occur in anoxia. Changes in perinatal and adult brain neurotransmitter content and release may be related to clinical impairment of cognition. H_2S exposures at concentrations below the current occupational limits cause physiological changes in pulmonary function, thus suggesting that asthmatics are at risk. Studies of fetal and neonatal brain tissue have shown an abnormal development, and the long-term consequences of these neuronal changes have not yet been assessed. Finally, new approaches to therapy are required, such as the use of agents that actively remove sulfide from its sites of action. This may prove more useful in preventing some of the long-term adverse sequelae than the use of nitrites and hyperbaric O_2, although the latter should be used in cases of pulmonary edema.

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