The Role of Hydrogen Sulfide in Evolution and the Evolution of Hydrogen Sulfide in Metabolism and Signaling

The chemical versatility of sulfur and its abundance in the prebiotic Earth as reduced sulfide (H\textsubscript{2}S) implicate this molecule in the origin of life 3.8 billion years ago and also as a major source of energy in the first seven-eighths of evolution. The tremendous increase in ambient oxygen ~600 million years ago brought an end to H\textsubscript{2}S as an energy source, and H\textsubscript{2}S-dependent animals either became extinct, retreated to isolated sulfide niches, or adapted. The first 3 billion years of molecular tinkering were not lost, however, and much of this biochemical armamentarium easily adapted to an oxic environment where it contributes to metabolism and signaling even in humans. This review examines the role of H\textsubscript{2}S in evolution and the evolution of H\textsubscript{2}S metabolism and signaling.

The simplest definition of life is the ability to utilize and control energy. Today, nearly all of life’s energy is derived from the sun. Plants oxidize water to oxygen and reduce inorganic carbon, whereas animals derive energy by reversing this process. Photosynthesis was not an innate property when life originated, and a number of scenarios have been proposed to provide energy and/or energized organic molecules. Hydrogen sulfide (H\textsubscript{2}S) is mentioned in most scenarios, but generally as a minor contributor. In this review, we will present arguments suggesting that H\textsubscript{2}S had a far greater role in the origin of life and primordial metabolism than previously thought. Remnants of these activities persist in modern animals, not as a primary energy source, but as an important regulator or modulator of metabolism and signaling.

Sulfur and Sulfide Chemistry

Sulfur is the 10th most common element in the universe, the 15th most common in the Earth’s crust, and the 7th most common element in animals (53). This biological concentration is indicative of sulfur’s considerable utility and versatility in living systems. Sulfur has eight formal oxidation states, \(-2\) to \(+6\), with even integers being the most stable. H\textsubscript{2}S (\(-2\)), the most reduced, is a weak acid; H\textsubscript{2}S \(\leftrightarrow\) HS\textsuperscript{\(-\)} + H\textsuperscript{+}, where pKa\textsubscript{1} is 6.9 and pKa\textsubscript{2} is between 12 and 17 (119). At pH 7.0, dissolved H\textsubscript{2}S \(\sim\) HS\textsuperscript{\(-\)}, whereas S\textsuperscript{2\textsuperscript{-}} is often considered to be essentially negligible, the latter a mistake that ignores the fact that, in an equilibrium, S\textsuperscript{2\textsuperscript{-}} can theoretically be generated until all sulfide (H\textsubscript{2}S, HS\textsuperscript{\(-\)}, and S\textsuperscript{2\textsuperscript{-}}) is consumed (75). In cells, the HS\textsuperscript{\(-\)}-to-H\textsubscript{2}S ratio can change from 12.6 in the mitochondrial matrix (pH 8.0) to 0.006 in acidic lysosomes (pH 4.7). Dissolved H\textsubscript{2}S is lipophilic and readily diffuses through membranes (88), essentially creating pH-dependent equilibria on both sides of these barriers; however, ionized species are more chemically reactive. The temperature dependency of the pKa\textsubscript{1} can be described by the equation pKa\textsubscript{1} = 3.122 + 1,132/T, where T = degrees Kelvin (119). When life began, it is likely that the percent H\textsubscript{2}S, HS\textsuperscript{\(-\)}, and S\textsuperscript{2\textsuperscript{-}} in the deep open ocean (~2°C, pH 6.5) would have been 66, 33, ~0%; compared with 56, 43, ~0% in effluent from hot (400°C) acidic (pH 4.5) thermal vents (black smokers) or 4, 94, 1% in cooler (70°C) alkaline (pH 9.5) white smoker thermal vents. Dissolved H\textsubscript{2}S is also volatile, reflected by its 5-min half-life in open tissue culture wells, 3 min in aerated myographs, and <1 min in Langendorff perfused heart preparations (24). Nevertheless, its downstream biological effects can persist for hours. Perhaps the greatest single obstacle in the field of H\textsubscript{2}S biology is the accurate measurement of intracellular H\textsubscript{2}S (77, 121).

A one-electron oxidation of two sulfides or a two-electron oxidation of one of the two sulfides forms the simple persulfide, H\textsubscript{2}S\textsubscript{2\textsuperscript{-}}. Additional oxidative steps form progressively longer polysulfide chains, up to S\textsubscript{n}, at which point the sulfur chain is presumed to cyclize and become insoluble (169), although this is not always the case (see below). Polysulfides can act as either a reductant or an oxidant, a point considered in greater detail later. pKa\textsubscript{1} and pKa\textsubscript{2} for H\textsubscript{2}S\textsubscript{n} rapidly decreases as n increases (60), potentially increasing reactivity.
A Brief History of the Earth

The earth was formed ~4.6 billion years ago (bya), and it is defined by four eons. The Hadean Eon, named after Hades, was inhospitable, excessively hot, and anoxic. If life began here, it would have been destroyed by extraterrestrial impacts of unimaginable magnitude and frequency, but these would have also brought life’s essentails, water, an atmosphere, and organic molecules (156). Life began early in the Archean eon (3.8 bya; FIGURE 1) in a warm and ferrungous (anoxic and Fe2⁺ dominated) ocean (138, 149). The Proterozoic eon began 2.5 bya. Oxygen appeared in the atmosphere ~2.3 bya, the “great oxidation event” (GOE) in which atmospheric oxygen may have increased several times to ~2% while the oceans remained essentially anoxic. Evolution of modern-day plants, some 600 million years ago (mya), ushered in the Phanerozoic eon and the tremendous biomass that could only be supported by solar energy and an abundance of atmospheric oxygen.

Origin of Life

Theories of life’s origin follow two main themes: Where did the first organic molecules come from and how was energy harnessed to drive metabolism? Stanley Miller was the first to suggest that lightning could have provided the energy to create the “primordial soup” (98). Other sources of organic molecules include high-energy nuclear reactions in far-off stars then delivered in comets, meteors, or cosmic dust (127, 130, 131), and photocatalyzed reactions in the atmosphere (147). H₂S is present in all of these possibilities, even in recently discovered samples from Miller’s original experiments (126, 127). While all of these theories provide organic precursors, they cannot consistently deliver useful energy, and dispersion of the initial products in the ocean or atmosphere limits the probability of coupled, sequential chemical reactions. Thus recent attention has turned to hydrothermal vents. In fact, the prebiotic earth has been likened by some to a prototypical cell where energy in the form of reducing equivalents traverses these vents as chemiosmotic gradients do across a cell membrane (89, 136).

Hydrothermal vents are created along the separation lines of tectonic plates, e.g., the mid-ocean ridge-spreading centers. There are two general types, black and white smokers, so named for the color of the vent effluent. Black smokers are close to the spreading centers where magma heats seawater that has seeped into the crust and they emit hot (300–400°C), acidic (pH 2–3) seawater rich in CO₂ (4–215 mm/kg), H₂S (3–110 mmol/kg), dissolved H₂ (0.1–50 mmol/kg), and reduced transition metals, especially iron (Fe²⁺). Iron and sulfide react in the vent fluid to form FeS, which is then precipitated when it contacts oxygenated seawater, thereby forming the characteristic black particulate plume (64, 82, 144). The combination of high pressure and heat can drive reactions not kinetically possible under other conditions, and when both temperature and pressure decrease as these fluids rise from near the magma toward the seafloor, the stability of more thermally labile products is favored. Heat deep within the smokers keeps metal sulfides in solution, and acidity favors their dissociation and elevates H₂S concentrations (51). In the prebiotic earth, it is quite likely that H₂S and reduced metal sulfides remained in solution in anoxic seawater for prolonged periods and could have spread considerable distances (35). Present-day vents contain the densest biomass on earth, evidence of their abundant energy and the ability of living organisms to use that energy.

The recently discovered white smokers are typically found lateral to the mid-ocean Ridge (off-axis vents) and are alkaline (pH 9–11) and cooler (70–90°C), as magmatic heating is considerably reduced (82, 150). They have high concentrations of H₂ (<1–12 mM) and CH₄ (1–2 mM), but little CO₂ or H₂S. White smokers sit on or near the magnesium- and iron-rich mineral olivine, which, when in contact with seawater, creates an exothermic reaction ultimately generating H₂ through a process known as serpentinization (145). The heat generated by this process also drives a hydrothermal circulation (145). Much recent work has focused on this process as providing the energy and the chemistry for the origin of life in the form of reducing equivalents (H₂) that can then form methane from CO₂ and by creating a chemio-osmotic gradient between alkaline the vent fluid and circumneutral (pH 6.5) seawater (10, 23, 48, 82, 83, 85–87, 89, 113–115, 142, 143, 145, 146, 150, 157, 173, 180). Paradoxically, white smokers support relatively little biomass or diversity (145).

Some vents are more unique and provide evidence for H₂S in life’s origins. These vents are found on or near tremendous deposits of metal sulfides, often called sulfide lenses (152, 153). They are relatively hot (200–370°C) because they are heated by both magmatic flow and serpentinization, acidic (pH 3–4), and with high concentrations of H₂S (0.5–2 mM), H₂ (10–25 mM), and CH₄ (0.5–2.5 mM). It is our opinion that these events offer the greatest opportunity for life due to the versatility of sulfide and the many energetic transformations that can occur.
The Multifunctional Role of \( \text{H}_2\text{S} \) at Life’s Origin

\( \text{H}_2\text{S} \) was arguably the most versatile molecule when life began because it could serve as an important organic product, reactant, catalyst (pro-enzyme), barrier (proto-membrane), and sustainable source of energy. In the “iron-sulfur world” (172), oxidation of \( \text{HS}^- \) by \( \text{FeS} \), both products of hydrothermal vents, produces a variety of organic molecules (reviewed in Ref. 16) as well as reducing \( \text{N}_2 \) or nitrate to ammonia and generating amines (9, 27, 63, 116, 162). Sulfide reacts with \( \text{Fe}^{2+} \) and other transition metal ions, and many of these can serve as unique and gateway catalysts (22, 43, 104, 112, 116, 137). For example, sphalerite (ZnS) is a highly specific catalyst for activation of single carbon-hydrogen bonds (155). Sulfide and iron combinations form minerals such as pyrite (FeS2), greigite Fe3S4 (138, 140), and iron sulfur clusters such as Fe2S2 and Fe4S4, all of which cannot only act as catalysts but potentially act as physical barriers forming prototypical membranes (89, 92, 93). Iron sulfur clusters are also found in a variety of enzymes and act as chemical “wires” to conduct electrons; 12 are found in mitochondria. Transition metals also react with sulfur to form metal polysulfides, which increases sulfur’s reactivity and versatility.

A number of factors support \( \text{H}_2\text{S} \) over \( \text{H}_2 \) as the primordial energy source. First, there is typically more \( \text{H}_2\text{S} \) than \( \text{H}_2 \) exhausted from vents. Second, transition metal sulfides (e.g., FeS) can potentially release more \( \text{H}_2\text{S} \) per volume from sulfide lenses (55,000 mol/m3) than \( \text{H}_2 \) can be generated from olivine (500 mol/m3 olivine; Ref. 82). Third, oxidation of \( \text{H}_2\text{S} \) produces more energy than \( \text{H}_2 \) oxidation

\[
\text{H}_2\text{S} + 4\text{H}_2\text{O} \rightarrow \text{H}_2\text{SO}_4 + 4\text{H}_2 : -662.7\text{kJ/mol} \tag{1}
\]

vs.

\[
4\text{H}_2 + \text{CO}_2 + \text{CH}_4 + 2\text{H}_2\text{O} : +343.8\text{kJ/mol} \tag{2}
\]

or

\[
\text{H}_2\text{S} + 2\text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{H}_2\text{SO}_4 + \text{CH}_4 : -318.9\text{kJ/mol} \tag{3}
\]

Fourth, \( \text{H}_2\text{S} \) oxidation generates additional equivalents of \( \text{H}_2 \) (Eq. 1). And fifth, complete oxidation of \( \text{H}_2\text{S} \) to \( \text{H}_2\text{SO}_4 \) releases eight electrons, enough to completely reduce carbon to methane compared with two electrons released by \( \text{H}_2 \) oxidation.

**\( \text{H}_2\text{S} \) and Photosynthesis**

The ability to extract energy from a photon and use it to form or break chemical bonds freed organisms from their chemolithotrophic existence and their dependency on reducing equivalents supplied from within the Earth to drive cellular redox chemistry. This likely occurred relatively soon after the origin of life ~3–3.5 bya (49, 149, 156). The initial type-I photosynthetic pathways were sequential two-electron transfer processes mediated by soluble cytochromes and were anoxicogenic. Their light-gathering antennae absorbed longer wavelength light and, because water is a weak electron donor, reduced compounds, such as \( \text{H}_2\text{S}, \text{H}_2, \text{Fe}^{2+} \), organic carbon, and nitrate, have been suggested as possible electron sources (139). \( \text{H}_2\text{S} \) would not only be a likely candidate because of its abundance, but the molecular similarity to water would be a convenient “lead-in” to more sophisticated high-energy type-II photosynthesis that followed. Relics of \( \text{H}_2\text{S} \)-mediated photosynthesis are present in modern-day anerobic photosynthetic purple and green sulfur bacteria as

\[
\text{CO}_2 + \text{H}_2\text{S} + \text{hv} \rightarrow (\text{CH}_2\text{O})_n + \text{H}_2\text{O} + \text{S}(\alpha) \tag{4}
\]

where \( S(\alpha) \) denotes polysulfides or elemental sulfur that is formed and packed into globules that are either excreted or retained within the cell. The latter may still be important as a means of sulfur storage, trafficking, and signaling as discussed in the last section. Perhaps bespeaking to their primal origins, some extant green anoxicogenic photosynthetic bacteria have light-gathering antennae, chlorosomes, tuned to the low-energy infrared radiation emitted from hydrothermal vents (7). As in mitochondria, Fe2S2 clusters also assist in electron transfer in chloroplasts (123).

Oxygenic photosynthesis, a four electron oxidation of two water molecules, first appeared in cyanobacteria probably several hundred million years after anoxicogenic photosynthesis. This “great oxidation event” (GOE) may have periodically increased atmospheric oxygen to <2% (Po2 of <15 Torr) of present atmospheric levels (pal) ~2.3 bya (19, 28, 50, 149). However, the oceans remained largely anoxic, and recent studies suggest that atmospheric oxygen levels were considerably lower than previously suggested, at most 0.1% of pal (Po2 of <2 Torr) even from 1.8 to 0.8 bya (132). Because the light-gathering antennae of primitive anoxicogenic chlorophyll (bacteriochlorophyll) could not collect sufficient energy to oxidize water, it has been proposed that other intermediates such as hydroxyl amine, hydrogen peroxide, hydrazine, nitric oxide, nitrite, or HCO3 were the “transitional” electron donors leading up to oxygenic processes (49). Raymond and Blankenship (139) suggest that hydrogen peroxide was the most likely intermediate and propose that binuclear manganese catalase ultimately became the tetranuclear manganese on the oxygen evolving complex (OEC) of chlorophyll.
We propose that \( H_2S \) or hydrogen persulfide \( (H_2S_2) \) would be better “transitional” electron donors than peroxide. The oxidation potential for \( H_2S \rightarrow S^0 + 2H^+ + 2e^- \) is \(-0.14 \text{E}^{0}(V)\), far less than that of water to peroxide; \( 2H_2O \rightarrow H_2O_2 + 2H^+ + 2e^- \) \([-1.78 \text{E}^{0}(V)] \) or peroxide to oxygen \( H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \) \([-0.68 \text{E}^{0}(V)] \). There was also considerably more \( H_2S \) in the environment than \( H_2O_2 \). Using \( H_2S \) would also provide a logical transition where \( H_2S_2 \) derived from two-electron oxidation of \( H_2S \) in anoxygenic photosynthesis could be utilized in a second reaction with \( H_2S \), e.g.

\[
H_2S + h\nu \rightarrow 2H^+ + 2e^- + S^0, \text{ then; } S^0 + H_2S \rightarrow H_2S_2 \tag{5}
\]

forming progressively longer chain polysulfides. In addition, \( H_2S \) could easily have been the antecedent four-electron donor paving the way for its co- 

gener, water

\[
2H_2S + h\nu \rightarrow 4H^+ + 4e^- + S_2 \tag{6}
\]

**Sulfide and the Origin of Mitochondria**

The slight increase in atmospheric oxygen during the GOE oxidized terrestrial sulfur to sulfate, which was then washed to the sea. Here, the omnipresent Fe\(^{2+}\), along with the appearance of a few sulfate-reducing organisms (65), reduced sulfate to \( H_2S \), and large areas of ocean became euxinic (anoxic and sulfidic). Eukaryotes first appeared in this environment. The following paragraphs describe the evolution of organisms and metabolic mechanisms that oxidize sulfide; organisms that reduce sulfite and sulfate back to sulfide are considered elsewhere (5, 6).

Eukaryotes require mitochondria to transform oxygen reduction into useful energy. It is most often accepted that mitochondria are derived from a single endosymbiotic event \(~1.5\) bya in which their precursor, an \( \alpha \)-proteobacteria akin to *Rickettsia*, was engulfed by a host Archea (21, 28, 81, 165, 178). A novel monophyletic archael phylum “Lokiarchaeota” with genes coding numerous eukaryotic signature proteins is a likely ancestral host (158). Not surprisingly, Lokiarchaeota were found in sediment near the black smoker hydrothermal vent, Loki’s Castle (158). A number of advantages have been attributed to such a union. For instance, the “Ox-Tox” model suggests this union prevents oxygen toxicity (72), although an intracellular organelle is not ideally suited to protect the cytosol from extracellular insult. The “hydrogen” hypothesis suggests this as a mechanism of hydrogen transfer (84), although loss of hydrogen from the atmosphere could be problematic. On the other hand, “sulfide syntrophy” (151) suggests a mechanism of sulfur cycling. This is intriguing since it incorporates features of a sulfide-reducing host with the sulfide-oxidizing endosymbiont, an advantageous union in the euxinic ocean where sulfide could provide energy. Sulfur syntrophy is also consistent with sulfur cycling in modern-day eukaryotes (see below), and it reflects the primordial lineage of sulfide-metabolizing enzymes, including some organisms with anaerobic mitochondria (91).

The first three steps in \( H_2S \) metabolism in humans and some bacteria are identical, suggesting a long phylogenic relationship (90). Indeed, the enzyme sulfur quinone oxidoreductase (SQR), the first step in \( H_2S \) metabolism (see below), not only appears to have been present in the original mitochondrial endosymbiont (167), it is physically embedded in the eukaryotic electron transport chain of extant animals (47). Because many elements of the mammalian electron transport chain as well as SQR predate the emergence of cyanobacteria, and therefore predate oxigenic photosynthesis (12, 13, 39), it seems reasonable to conclude that these systems initially served another energetic pathway, and \( H_2S \) oxidation would be the most logical candidate.

**The Advent of Environmental Oxygen, Demise of Free Sulfide, and Origin of Modern-Day Animals**

Subsequent endosymbiotic events in which eukaryotic cells incorporated cyanobacteria gave rise to modern plants at the beginning of the Phanerozoic (FIGURE 1), \(~800\) mya (4, 34, 50, 61, 62, 71, 79, 138, 149, 179). The combined activity of cyanobacteria and plants tremendously increased oxygen production, but the oxygen was quickly “mopped up” by the vast amounts of reduced iron and sulfide. This probably took another several hundred million years, but, when finished, the oceans were oxidized, atmospheric oxygen rose to present-day values, and sulfide was effectively eliminated as an energy source. It is generally thought that the rise in oxygen posed a new threat to life, i.e., organisms either developed antioxidant mechanisms to deal with oxygen’s toxic effects, retreated to anoxic environments, or became extinct. However, we propose an alternative explanation. Because antioxidant mechanisms were already in place to deal with reactive sulfide species (RSS), they needed to be only slightly tuned to deal with reactive oxygen species (ROS). This allowed animals access to the practically unlimited supply of reduced carbon compounds now provided by plants and to the most potent and abundant electron acceptor, oxygen. The result was a massive explosion in Earth’s biomass and complexity.
Sulfide Metabolism in Modern-Day Metazoans

For all practical purposes, the rise in oxygen 600 mya divided eukaryotes into two groups, phototrophs and chemotrophs, the former producing oxygen and reducing inorganic compounds, mainly those of carbon, and the latter, basically consumers, completely dependent on the former’s activities. Assimilation and reduction of oxidized sulfur (mainly sulfate and sulfite) by microorganisms and plants can be found in recent reviews (31, 49, 141) and will not be considered here. Metazoans in general, and vertebrates in particular, which will be considered in detail, typically cannot reduce sulfur compounds more oxidized than S(−2). Thus animals must rely on plants and prokaryotes for these compounds, nearly all of which are incorporated as completely reduced S(−2) sulfur amino acids (S-AA), methionine (the only essential S-AA), and cysteine. For instance, most of the human sulfur intake in Western societies is used for synthesis. The average intake of S-AA is 26 mmol/day, and S-AA from protein turnover adds another 70 mmol/day, ~90% (88 mmol/day) of which is used for protein synthesis (53, 54). Although gut flora produces considerable H₂S, up to 40 μM in the colon, it is effectively oxidized by the epithelium and is not an appreciable source of reduced sulfur (29, 78). The general features of sulfide synthesis and metabolism are shown in FIGURE 2.

H₂S Production

H₂S can be generated via a number of mechanisms from l-homocysteine and l-cysteine via the methionine transsulfuration pathway or from dietary cysteine (15, 58, 160). H₂S can also be formed by reduction of sulfur in persulfides, a process well characterized in protozoans but only recently receiving attention in vertebrates (discussed below). Two enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE aka CGL), are found in the cytosol, and the tandem of cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfurtransferase (3-MST) are found in the cytosol and in the mitochondrial matrix (59, 94, 107). There are also differences in enzyme distribution, CBS predominates in neural and CSE in cardiovascular

**FIGURE 1.** Time line of evolution relative to atmospheric oxygen (O₂, blue line) and oceanic H₂S (orange line)

Other than possibly a few “whiffs,” atmospheric O₂ was essentially nil from the onset of life ~3.8 billion years ago (bya) until the great oxidation event (GOE) 2.3 bya, the latter correlating with a substantial rise in H₂S. Eukaryotes first appeared 1.5 bya in anoxic and sulfidic (euxinic) oceans and developed for hundreds of millions of years until O₂ production by oxygenic cyanobacteria and plants oxidized the H₂S and Fe²⁺ ~0.6 bya, essentially eliminating sulfide as an energy source. Mass extinctions (*) were often associated with a fall in ambient O₂ and increase in H₂S, perhaps providing a biological filter for descendants that retained some degree of tolerance to hypoxia and sulfide.
H$_2$S can also simply diffuse out of cells, but most synthesize H$_2$S by taking advantage of the three responses, as well as abundant CAT and 3-MST, can oxidase, thus increasing mitochondrial CBS. Both degradation, is no longer catabolized during hypoxia, translocate CSE from the cytosol to the mitochondria, whereas CBS, which is normally translocated to the mitochondrion for degradation, is no longer catabolized during hypoxia, thus increasing mitochondrial CBS. Both responses, as well as abundant CAT and 3-MST, can synthesize H$_2$S by taking advantage of the three-fold greater cysteine concentration in the mitochondrial matrix compared with the cytosol (32, 166).

However, under normal circumstances, the overall flux of sulfur into the transsulfuration pathway, and hence H$_2$S production, may be relatively constant. In the presence of oxygen, cysteine dioxygenase (CDO) irreversibly oxidizes cysteine to cysteinesulfinate (and ultimately to hypotaurine or sulfate/sulfate), thereby decreasing S-AA flux through the transsulfuration pathways. CDO activity and expression can increase some 450-fold in response to increased dietary cysteine. Thus as little as 35% of cysteine sulfur is oxidized by CDO in low-cysteine diets, whereas this can increase to 97% when cysteine is in great excess. In this capacity, CDO may serve as a biological “safety valve” setting fairly tight limits on H$_2$S production (161).

H$_2$S Catabolism

Of the three transmitters, CO, NO, and H$_2$S, only the latter is enzymatically inactivated. Chemotrophic and phototrophic microorganisms can oxidize sulfide via a number of different pathways, including sulfur quinone oxidoreductase (SQR), flavocytochrome c sulffate dehydrogenase (Fcc), and the sulfur oxidizing (SOX) pathway, and this can be accomplished aerobically or anaerobically, the latter using nitrate as the electron acceptor (40, 134, 148)

$$\text{HS}^- + 1.6\text{NO}_3^- + 0.6\text{H}^+ \rightarrow \text{SO}_4^{2-} + 0.8\text{N}_2 + 0.8\text{H}_2\text{O} \quad (7)$$

H$_2$S can also simply diffuse out of cells, but most evidence suggests that, in eukaryotes, H$_2$S is inactivated in mitochondria (118). Vertebrates have SQR but neither Fcc nor SOX pathways. Although it is often stated that only prokaryotes use reduced sulfur as electron donors for respiration (148), this is clearly not the case, and a variety of metazoans including invertebrates, fish, birds, and mammals can generate ATP from mitochondrial sulfide oxidation (2, 25, 26, 36, 128, 135, 171, 177).

Vertebrates and invertebrates share common pathways for oxidizing H$_2$S, although there are still some uncertainties, even in mammals (8, 36, 46, 47, 56, 73, 80, 90, 167). There is general agreement that in the initial step H$_2$S binds to the SQR enzyme and is oxidized to sulfane sulfur (S) forming persulfide (SQRS-S). This also transfers two electrons via FAD into the quinone pool. These electrons are carried via the electron transport chain to complex III and IV, and the chemiosmotic gradient derived from this drives ATP synthesis. There are differing thoughts on the disposition of the SQR-sulfane sulfur. The Jorns group (56, 90) proposed that sulfane sulfur is first transferred to sulfite ($\text{SO}_4^{2-}$) forming thiosulfite ($\text{S}_2\text{O}_3^{2-}$; FIGURE 2, reaction 1) and then to glutathione (GSH) forming glutathione persulfide (GSSH). Thiosulfate:glutathione sulfur transferase (TST) supposedly catalyzes the latter step. Although TST has not been identified in mammals, its gene (TSTD1, thiosulfate sulfurtransferase rhodanase-like domain containing 1), homologous to its yeast ortholog RDL1, recently has been identified. TST is not a rhodanase. The mitochondrial sulfur dioxygenase (SDO, aka ETHE1) then oxidizes sulfane sulfur of GSSH to sulfite, consuming O$_2$ and H$_2$O in the process. Sulfite can be further oxidized by sulfite oxidase (SO) to sulfate ($\text{SO}_4^{2-}$), resulting in liberation of 2H$^+$ and 2e$^-$, the latter transferred to cytochrome c (57) also contributing to ATP production. Alternatively, sulfite can be metabolized by SQR with an additional H$_2$S to form thiosulfate. Based on kinetic analysis, Libiad et al. (76) proposed an alternative pathway where GSH receives the SQR sulfane sulfur, forming GSSH (FIGURE 2, reaction 2). GSSH is then oxidized by SOD (ETHE1) to $\text{SO}_4^{2-}$, and the GSH recovered. $\text{SO}_4^{2-}$ then can be oxidized to $\text{S}_2\text{O}_3^{2-}$ by SO, or rhodanase (Rhd) can catalyze sulfur transfer from GSSH, producing $\text{S}_2\text{O}_3^{2-}$. H$_2$S can also be recovered from $\text{S}_2\text{O}_3^{2-}$ by endogenous reductants dihydrolipoic acid (DHLA) or thioredoxin (Trx; reaction 3; Refs. 94, 120).

In addition to directly stimulating ATP production by donating reducing equivalents to the electron transport chain, H$_2$S inhibits mitochondrial phosphodiesterase 2A, and the resultant increase in cAMP will further stimulate electron transport (103). ATP production from H$_2$S has been proposed to balance Krebs cycle-derived electron donors and, by enhancing mitochondrial bioenergetics, helps protect against a variety of stressors (reviewed in Refs. 101, 163). The advent of mito-
chondrial-targeted H₂S-releasing drugs (164) should permit considerable insight into this field.

**H₂S Toxicity**

The hormetic effect of H₂S is well known; at low concentrations H₂S stimulates O₂ uptake and ATP production, whereas these reactions are inhibited at higher H₂S concentrations through H₂S inhibition of cytochrome c-oxidase (COX). Purified COX is reversibly inhibited by as little as 0.2 μM H₂S, whereas progressively higher concentrations (up to 20–40 μM) are needed to inhibit oxygen consumption by mitochondria and intact cells (1, 8, 17, 102, 129). Thiosulfate is often the excretory product of organisms inhabiting sulfidic and hypoxic environments, since excretion of two sulfur atoms requires only three oxygen atoms, whereas sulfate is normally excreted by animals in normoxic environments (20, 26, 41). SQR activity is generally correlated with increased resistance to H₂S toxicity, and it is increased to offset an increased H₂S load; sulfate-synthesizing enzymes are concomitantly decreased as O₂ availability decreases (33, 42, 44, 55, 74, 97). In acute hypoxia, H₂S may be detoxified by reversing electron flow and reducing fumarate to succinate (36, 41). This has been pro-

**FIGURE 2.** Pathways for H₂S production and catabolism in vertebrates

H₂S synthesis: in the cytosolic transsulfuration pathway, homocysteine generated from methionine can directly, or in combination with L-cysteine, produce H₂S catalyzed by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). H₂S can also be produced directly from dietary cysteine. Cysteine dioxygenase (CDO) maintains intracellular cysteine concentrations, and potentially H₂S production, relatively constant by oxidizing excess cysteine to cysteine sulfonate, which then becomes sulfite (S₂O₃²⁻) and hypotaurine. CBS and CSE can also be translated into the mitochondria to take advantage of threefold higher cysteine concentrations in the matrix. Cysteine aminotransferase (CAT) catalyzes the formation of 3-mercaptopyruvate from cysteine, which then forms a persulfide with the enzyme 3-mercaptopyruvate sulfur transferase (3-MST) in both cytosol and mitochondria. H₂S can presumably be released from 3-MST-SH by another reductant such as thioredoxin (Trx) or dihydrolipoic acid (DHLA). D-Amino acid oxidase (DAO) in brain and kidney peroxisomes can also generate 3-MP from d-cysteine. H₂S catabolism: H₂S binds to the enzyme sulfur quinone oxidoreductase (SQR), forming a persulfide (SQR-S₂), in the process transferring two electrons via a quinone into the electron transport chain. These electrons ultimately are delivered to oxygen, and ATP is produced. In path 1, the sulfane sulfur is first transferred to the mobile carrier sulfite (S₂O₃²⁻), forming thiosulfate (S₂O₅²⁻), and then to glutathione (GSH) by thiosulfate sulfur transferase (TST), forming glutathione persulfide (GS-SH). Mitochondrial sulfur dioxygenase (ETHE1) oxidizes GS-SH to sulfite, which can then be further oxidized by sulfite oxidase (SO) to sulfate (SO₄²⁻) producing electrons that are delivered to cytochrome c (Cyt c) or receive another H₂S and form thiosulfate. Pathway 2 is similar except that GSH is the initial mobile carrier and rhodanase (Rhd) catalyzes formation of thiosulfate from sulfite and GSSH. H₂S can also be regenerated from thiosulfate by endogenous reductants dihydrolipoic acid (DHLA) and thioredoxin (Trx). An alternative oxidase (AOX) that accepts electrons from SQR but is not coupled to ATP production is found in invertebrates.
Dissolved H2SO4 and H2S-metabolizing enzymes. How endogenous H2S in the following section provides physiological benefits, including cytoprotection, anti-inflammatory, neuror modulation, and cardiovascular function (reviewed in Refs. 14, 68, 133, 174). These studies are based largely on the effects of exogenous H2S administration or after manipulation of H2S-metabolizing enzymes. How endogenous H2S is regulated is unclear. H2S also has been proposed to be an oxygen sensor (117). In this instance, it is clear that H2S concentration can be tightly regulated by the balance between constitutive H2S production through transsulfuration and the amount of oxygen available for its metabolism. The protective effects of H2S in a variety of models of ischemia (133) likely reflect a similar mode of oxygen-dependent H2S metabolism.

Four mechanisms of H2S signaling have been identified thus far. 1) Although supraphysiological concentrations of H2S inhibit mitochondrial COX, lower (and presumably physiological) concentrations contribute to energy production and mitochondrial stability (8, 36, 101, 163). Separating physiological from toxicological effects is an ongoing difficulty. 2) Completely reduced H2S sulfur (~2) can act as a reductant, and this appears to be a highly specific process for certain disulfides (170). Further identification of these disulfides and their proximity to H2S production should greatly enhance our understanding of H2S signaling. 3) Dissolved H2S or HS− can coordinate with or reduce iron in heme proteins. This has recently been described in a variety of complex reactions that regulate activity of heme peroxidases, such as myeloperoxidase and catalase (110, 124). 4) Perhaps the most interesting signaling mechanism is sulfhydration (more appropriately termed sulfurization). Two-electron oxidation of either H2S or cysteine sulfur (or a one-electron oxidation of both) forms sulfane sulfur, S° (168), which can react with a variety of other sulfur atoms in proteins and low molecular weight molecules to form persulfides and polysulfides. These are described in the following section.

**H2S and Sulfur Signaling**

Numerous homeostatic functions have been proposed for H2S, including cytoprotection, anti-inflammation, neuromodulation, and cardiovascular function (reviewed in Refs. 14, 68, 133, 174). These studies are based largely on the effects of exogenous H2S administration or after manipulation of H2S-metabolizing enzymes. How endogenous H2S is regulated is unclear. H2S also has been proposed to be an oxygen sensor (117). In this instance, it is clear that H2S concentration can be tightly regulated by the balance between constitutive H2S production through transsulfuration and the amount of oxygen available for its metabolism. The protective effects of H2S in a variety of models of ischemia (133) likely reflect a similar mode of oxygen-dependent H2S metabolism.

Four mechanisms of H2S signaling have been identified thus far. 1) Although supraphysiological concentrations of H2S inhibit mitochondrial COX, lower (and presumably physiological) concentrations contribute to energy production and mitochondrial stability (8, 36, 101, 163). Separating physiological from toxicological effects is an ongoing difficulty. 2) Completely reduced H2S sulfur (~2) can act as a reductant, and this appears to be a highly specific process for certain disulfides (170). Further identification of these disulfides and their proximity to H2S production should greatly enhance our understanding of H2S signaling. 3) Dissolved H2S or HS− can coordinate with or reduce iron in heme proteins. This has recently been described in a variety of complex reactions that regulate activity of heme peroxidases, such as myeloperoxidase and catalase (110, 124). 4) Perhaps the most interesting signaling mechanism is sulfhydration (more appropriately termed sulfurization). Two-electron oxidation of either H2S or cysteine sulfur (or a one-electron oxidation of both) forms sulfane sulfur, S° (168), which can react with a variety of other sulfur atoms in proteins and low molecular weight molecules to form persulfides and polysulfides. These are described in the following section.

**Polysulfide Production and Metabolism: the “Next Frontier”?**

Evidence is accumulating that polysulfides (RSnR, RSnH, H2S2n; n > 2) or persulfides (n = 2) may be the actual mediators of sulfide signaling (99, 110, 122, 125). These readily interact with regulatory protein cysteine sulfur and nitrogenous signaling species through a variety of mechanisms and can act as either an oxidant or a reductant (18, 38, 66, 67, 105, 111, 168, 169). It has been suggested that as much as 25% of protein cysteines in mammalian cells may have a sulfane sulfur associated with it (106).

Comparatively little is known about polysulfide metabolism in vertebrates, and most attention has focused on its role in H2S production and subsequent signaling. In the canonical pathway (FIGURE 2), cysteine metabolism by CAT and 3-MST generates the 3-MST persulfide (3-MST-S). Addition of a reductant such as thioredoxin or dihydrolipoic acid then releases H2S from the persulfide (69, 94, 108, 176). The sulfane sulfur (S) can also be transferred to another mobile thiol such as cysteine, homocysteine, or glutathione, e.g., 3-MST-S + RSH → 3-MST + RS-SH (176), and wend its way along to less mobile protein thiols (30, 122). Recently, Kimura’s group has shown that H2S3 can be formed directly from 3-MP by 3-MST and rhodanase in mammalian cells (70).

CSE and CBS catalyze the formation of a variety of cysteine hydro polysulfides (CysSSH, CysSSSH, and CysSSSSSH) and, secondarily, polysulfides (CysSSSSS, CysSSSSSSCys, CysSSSSSSSSCys) from cystine (CysSCys) in mammalian cells (FIGURE 3A; Ref. 52). Cystine is far more prevalent than cysteine or methionine in the oxidized extracellular environment, and it is readily transported into cells by the cystine/glutamate antiporter, system Xc− (11), or possibly the sodium-coupled neutral amino acid transporter (AT2; Ref. 52). This process can provide substantial sulfane sulfur in an intracellular store that may then be transferred to glutathione (GS−H and GS−G; n = 2–4) and act as an intracellular reductant or intracellular signal (52). Unlike H2S, where intracellular concentrations are expected to be in the low nanomolar range (121), high polysulfide concentrations can be achieved; glutathione persulfide has been estimated to exceed 100 μM (52).

Recycling polysulfides for H2S or energy production has yet to be examined in vertebrates, but it has been described in some prokaryotes, most notably phototrophic (green and purple) sulfur bacteria (FIGURE 3B; Refs. 31, 37). Sulfur generated in anoxygenic photosynthesis (Eq. 4) is stored in intracellular or extracellular sulfur globules. Interestingly, cyclization and precipitation as elemental sulfur (S0) is inhibited, and sulfur is retained as long (n > 3 and possibly up to n > 103), linear, and stable polymers. These can be further oxidized or reduced back to H2S if environmental H2S availability falls. This regeneration of H2S as an electron donor may be the antecedent of eukaryotic sulfur
cycling important for mitochondrial integrity or redox signaling.

Polysulfides may have another unappreciated link with evolution and our current concept of both toxicity and signaling with reactive oxygen species (ROS). Stepwise one-electron oxidation of H₂S (HS⁻) initially produces a thyl radical (HS·⁻; FIGURE 1C). Two of these can combine to produce hydrogen persulfide (H₂S₂), which then can be oxidized to a persulfide radical (S₂·⁻) and then to elemental sulfur (Sn). These intermediates, reactive sulfide species (RSS), are surprisingly chemically and biochemically similar to the ROS intermediates in one-electron reduction of oxygen (FIGURE 3D) or one-electron oxidation of water. However, RSS have been around since life originated and were probably very prevalent in early anoxicogenic photosynthesis. Conversely, ROS only became an appreciable physiological problem after oxygenic photosynthesis caused oxygen to be formed some 600 million years ago. Hydrogen peroxide (H₂O₂) has garnered most attention as a signaling ROS because of its relative stability, membrane permeability, and ability to selectively react with protein thiols (175). Hydrogen persulfide (H₂S₂) shares many of these characteristics with hydrogen peroxide but appears even more reactive than peroxide in inactivating the lipid phosphatase PTEN (38). It is quite likely that some of the perceived ROS signaling may in fact be RSS signaling. Our laboratory (DeLeon ER, Gao Y, Huang E, Arrif M, Arora N, Divietro A, Olson KR, unpublished observations) recently found that a number of methods historically used to measure ROS, including redox-sensitive green fluorescent protein (roGFP), 2’,7’-dihydrodichlorofluorescein (DCF), MitoSox Red, Amplex Red, and H₂O₂ amperometric electrodes, are as, or often more, sensitive to RSS than they are to ROS. How these findings impact our understanding of cellular oxidants, antioxidants, and redox signaling remains to be determined. Sorting this out is the “next frontier” in sulfide biology.

FIGURE 3. Polysulfide shuttling in mammals and green sulfur bacteria, and similarities between reactive sulfide species and reactive oxygen species

A: in mammals, cystine (Cys-SCys), abundant in plasma and extracellular fluid, is taken up by cells via the cystine/glutamate antiporter (system X̅c) or via the sodium-coupled neutral amino acid transporter (AT2). Cytosolic CBS and CSE then catalyze formation of cysteine (Cys) hydrosulfides and polysulfides [CysS-S(n)H and CysS-S(n)Cys, respectively] and Cys can be exchanged for glutathione (GSH or G). H₂S can be regenerated from the hydrosulfides and polysulfides by two electron reductants. Image is modified from Ref. 52 and is used with permission from Proc Natl Acad Sci USA.

B: generic mechanisms of polysulfide (PS) shuttling by phototropic green and purple sulfur bacteria. H₂S is taken up and oxidized by sulfur quinone:oxidoreductase (SQR) similar to eukaryotes, or flavocytochrome c (FccAB), and ultimately stored in an intracellular (not shown) or extracellular globule as linear polysulfides that can exceed 10⁵ sulfur molecules. The sulfide oxidation (SOX) pathway metabolizes thiosulfate via SOX enzymes (SoxAXX and SoxYZ) that also form polysulfides. Stored polysulfides can be recovered during low H₂S and H₂S regenerated by dissimilatory sulfide reductases (DsrL) using electrons from NADH. Image is modified from Ref. 37 and is used with permission from Front Microbiol.

C: stepwise one-electron oxidation of H₂S forms the thyl radical (HS·⁻), hydrogen persulfide (H₂S₂), persulfide radical (S₂·⁻), and elemental sulfur (Sn). D: stepwise one-electron reduction of O₂ forms superoxide (O₂⁻⁻), hydrogen peoxide (H₂O₂), hydroxyl radical (OH·), and water. Biologically important reactive oxygen species (in blue) are homologous to reactive sulfide species (in red).
The authors thank numerous colleagues for stimulating discussions and elucidating mechanisms of sulfide biology.

Supported in part by National Science Foundation Grants IOS 0641436, IOS 1051627, and IOS 1446310 (K.R.O.).

No conflicts of interest, financial or otherwise, are declared by the author(s).

Author contributions: K.R.O. and K.D.S. conception and design of research; K.R.O. interpreted results of experiments; K.R.O. prepared figures; K.R.O. and K.D.S. drafted manuscript; K.R.O. and K.D.S. edited and revised manuscript; K.R.O. and K.D.S. approved final version of manuscript.

References


57. Johnson-Winters K, Tollin G, Enemark. JH. Eluci-
dating the catalytic mechanism of sulfite oxidiz-
ing enzymes using structural, spectroscopic, and
kinetic analyses. Biochemistry 49: 7242–7254,
2010.

58. Kabil O, Vitvitsky V, Xie P, Banerjee R. The quan-
titative significance of the transsulfuration en-
zymes for H2S production in murine tissues.

59. Kamoun. P. Endogenous production of hydrogen
sulfide in mammals. Amino Acids 26: 243–254,
2004.

60. Kamovsky M, Goifman A, Rizkov D, Lev O.
Formation of carbonic sulfide by the reaction of
carbon monoxide and inorganic polysulfides.

61. Kasting JF. Earth’s early atmosphere. Science

62. Kasting JF, Catling DC, Zahnle K. Atmospheric
oxidation and volcanism. Nature 487: E1,
2012.

63. Keller M, Bloch E, Wachtershauser G, Stetter
KGO. Formation of amide bonds without a con-
 densation agent and implications for origin of

64. Kelly DS, Baross JA, Delaney JR. Volcanoes,
fl uids, and life at mid-ocean ridge spreading cen-
ters. Annu Rev Earth Planet Sci 30: 385–489,
2002.

65. Kim KM, Qin T, Jiang YY, Chen LL, Xiong M,
Caetano-Anolles D, Zhang HY, Caetano-Anolles
G. Protein domain structure uncovers the origin
of aerobic metabolism and the rise of planetary

66. Kimura H. Hydrogen sulfide and polysulfides as
biological mediators. Molecules 19: 16146–
16157, 2014.

67. Kimura H. Signaling molecules: hydrogen sulfide
and polysulfide. Antioxid Redox Signal 22: 362–
376, 2015.

68. Kimura H. Signaling of hydrogen sulfide and
polysulfide. Antioxid Redox Signal 24: 347–349,
2015.

signaling molecules in rat brain. FASEB J 27:

70. Kimura Y, Toyofuku Y, Koike S, Shibuya N, Na-
gahara N, Iler D, Osawa Y, Kimura H. Identifi-
cation of H2S2 and H2S produced by 3-mercapto-
pyruvate sulfurtransferase in the brain. Sci Rep 5:

71. Kump LR. The rise of atmospheric oxygen.

72. Kurland CG, Andersson SG. Origin and evolution
of the mitochondrial proteome. Microbiol Mol

73. Lagouette E, Mimoun S, Andriamihaja M, Chau-
montet C, Blachier F, Bouillad F. Oxidation of
hydrogen sulfide remains a priority in mammalian
cells and causes reverse electron transfer in
colonicocytes. Biochim Bioch Acta 1977:

74. Leschelle X, Goubourn M, Andriamihaja M, Blot-
tiere HM, Couplan E, Gonzalez-Barroso MD,
Pe t C, Pagniez A, Chaumontet C, Mignotte B,
Bouillad F, Blachier F. Adaptive metabolic re-
sponse of human colonic epithelial cells to the
adverse effects of the luminal compound sulfide.

75. Li Q, Lancaster JR Jr. Chemical foundations of
hydrogen sulfide biology. Nitric Oxide 35: 21–34,
2013.

76. Likoli M, Yadav PK, Vitvitsky V, Martinov M,
Banerjee R. Organization of the human mito-
chondrial hydrogen sulfide oxidation pathway.

77. Lin VS, Chen W, Xian M, Chang CJ. Chemical
probes for molecular imaging and detection of
hydrogen sulfide and reactive sulfur species in
biological systems. Chem Sens Rev 44: 4596–
4618, 2015.

78. Lindem DR. Hydrogen sulfide signaling in the
gastrointestinal tract. Antioxid Redox Signal 20:

79. Lyons TW, Reinhart CT, Planavsky NJ. The rise
of oxygen in Earth’s early ocean and atmosphere.

structure-based classification of sulfide:quinone

81. Margulis L. Archaeal-eubacterial mergers in
the origin of Eukarya: phylogenetic classification
of life. Proc Natl Acad Sci USA 93: 1071–1076,
1996.

82. Martin W, Baross J, Kelley D, Russell MJ. Hydro-
thermal vents and the origin of life. Nat Rev

83. Martin WJ, Russell MJ. On the origin of biochem-
istry at an alkaline hydrothermal vent. Philos
2007.

84. Martin WJ, Russell MJ. On the origins of cells:
a hypothesis for the evolutionary transitions from
abiotic geochemistry to chemoautotrophic pro-
karyotes, and from prokaryotes to nucleated
cells. Philos Trans R Soc Lond B Biol Sci 358:

85. Martin WJ. Early evolution without a tree of life.

86. Martin WF. Hydrogen, metals, bifurcating elec-
trons, and proton gradients: the early evolution
of biological energy conservation. FEBS Lett 586:

87. Martin WF, Sousa FL, Lane N. Evolution. Energy

88. Mathai JC, Missner A, Kugler P, Saparov SM,
Zeidel ML, Lee JK, Pohl P. No facilitator required
for membrane transport of hydrogen sulfide.
Proc Natl Acad Sci USA 106: 16633–16638,
2009.

89. McGlynn SE, Kanik I, Russell MJ. Peptide and
RNA contributions to iron-sulfur chemical gar-
dens as life’s first inorganic compartments, cata-
lysts, capacitors and condensers. Philos Trans A

90. Melideo SL, Jackson MR, Jorns MS. Biosynthesis
of a central intermediate in hydrogen sulfide me-
tabolism by a novel human sulfurate transferase
and its yeast ortholog. Biochemistry 53: 4739–
4753, 2014.

91. Mentel M, Martin WA. Anaerobic animals from
an ancient, anoxic ecological niche. BMC Biol 8:

92. Mielke RE, Robinson KJ, White LM, McGlynn SE,
McEachern K, Bhartia R, Kanik I, Russell MJ.
Iron-sulfur-bearing chimneys as potential catalytic
energy traps at life’s emergence. Astrobiology 11:
933–950, 2011.

93. Mielke RE, Russell MJ, Wilson PR, McGlynn SE,
Coleman M, Kidd R, Kanik I. Design, fabrication,
and test of a hydrothermal reactor for origin-of-
life experiments. Astrobiology 10: 799–810,
2010.

94. Mikami Y, Shibuya N, Kimura Y, Nagahara N,
Ogasawara Y, Kimura H. Thioreredoxin and dihy-
dropyridine acid are required for 3-mercaptopyr-
ivate sulfurtransferase to produce hydrogen

95. Mikami Y, Shibuya N, Kimura Y, Nagahara N,
Yamada M, Kimura H. Hydrogen sulfide prote-
s the retina from light-induced degeneration by
the modulation of Ca21 influx. J Biol Chem 286:
39379–39386, 2011.


