HHS Public Access

Author manuscript

Annu Rev Nutr. Author manuscript; available in PMC 2015 December 18.

Published in final edited form as:

Annu Rev Nutr. 2014 ; 34: 171–205. doi:10.1146/annurev-nutr-071813-105654.

Sulfur as a Signaling Nutrient Through Hydrogen Sulfide

Omer Kabil[*], **Victor Vitvitsky**[*], and **Ruma Banerjee**

Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan 48109-0600

Ruma Banerjee: rbanerje@umich.edu

Abstract

Hydrogen sulfide (H_2S) has emerged as an important signaling molecule with beneficial effects on various cellular processes affecting, for example, cardiovascular and neurological functions. The physiological importance of H_2S is motivating efforts to develop strategies for modulating its levels. However, advancement in the field of H_2S-based therapeutics is hampered by fundamental gaps in our knowledge of how H_2S is regulated, its mechanism of action, and its molecular targets. This review provides an overview of sulfur metabolism; describes recent progress that has shed light on the mechanism of H_2S as a signaling molecule; and examines nutritional regulation of sulfur metabolism, which pertains to health and disease.

Keywords

hydrogen sulfide; signaling; sulfur metabolism; nutrition; sulfide oxidation

INTRODUCTION

Sulfur was likely among the first nutrients used by ancient forms of life on Earth—along with other inorganic substrates such as H_2, CO, and N_2—for the production of energy and as building blocks of life. Sulfur-utilizing organisms can be divided into two groups: sulfur reducing and sulfur oxidizing (143, 188, 202). Sulfate-reducing microorganisms are anaerobic and include bacteria and archaea that obtain energy by coupling the oxidation of organic compounds or molecular hydrogen to the reduction of sulfate in a process known as sulfate respiration (24, 188). Other inorganic sulfur compounds can also serve as electron acceptors, including sulfite, thiosulfate, and elemental sulfur, producing hydrogen sulfide (H_2S) (24, 188). Sulfate-reducing bacteria are believed to be among the oldest microorganisms and can be traced back 3.5 billion years (24). Sulfur-oxidizing microorganisms include both aerobic and anaerobic organisms (91, 143, 188) and oxidize H_2S to elemental sulfur or further, to other oxidized sulfur compounds (e.g., sulfate) to generate reducing power.

[*]Equal contributors

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

Many invertebrate organisms thrive in sulfur-rich ecosystems and rely on sulfur-metabolizing endosymbionts to grow as well as to avoid the toxic effects of H_2S (13, 64, 65, 92). A well-studied example of such an invertebrate is the giant tube worm *Riftia pachyptila*, which lives in deep-sea hydrothermal vents that are sulfide rich (264). The ability to oxidize sulfide and its coupling to energy production is conserved in higher eukaryotes, and sulfide serves as an energy source for mammalian cells, providing reducing equivalents for the electron transport chain (271).

Mammals assimilate sulfur primarily from the diet in the form of the sulfur-containing amino acids methionine and cysteine, which are precursors for the synthesis of various sulfur-containing metabolites essential for cellular homeostasis. On the one hand, H_2S was long considered to be an environmental toxin produced by geochemical activity and by anaerobic organisms, including intestinal bacteria. The ability of animal tissues to degrade H_2S was viewed as a defense mechanism against intestinal or environmental exposure to H_2S. On the other hand, the therapeutic effects of sulfur springs have been known for several hundred years (52, 176, 195, 239). Curiously, although the ability of mammalian tissues to produce H_2S was known for a while, the possible physiological significance of this phenomenon did not get much attention until recently, with the discovery of the varied physiological effects of H_2S in systems including the cardiovascular, intestinal, and central nervous system (198, 210, 299).

METABOLISM OF SULFUR-CONTAINING AMINO ACIDS

Sulfur Metabolism

In addition to their utilization as building blocks for protein synthesis, the sulfur-containing amino acids methionine and cysteine are precursors for the synthesis of important sulfur metabolites such as H_2S and glutathione (GSH), the main cellular redox buffer (Figure 1). Methionine also provides cells with methyl groups that are used in various methylation reactions. The first step in methionine metabolism is its reaction with adenosine triphosphate (ATP), catalyzed by methionine adenosyltransferase, to produce *S*-adenosylmethionine (AdoMet), a universal methyl donor in transmethylation reactions. The transfer of the methyl group from AdoMet by a variety of methylases yields *S*-adenosylhomocysteine (AdoHcy), which is rapidly hydrolyzed to adenosine and homocysteine via the action of AdoHcy hydrolase. Homocysteine occupies a critical junction point in the sulfur network. It can be methylated to regenerate methionine or it can be used to synthesize cysteine and H_2S in the transsulfuration pathway. Remethylation of homocysteine by methionine synthase (MS), through transfer of a methyl group from 5-methyl tetrahydrofolate, completes the methionine cycle. In liver and in kidney, a second enzyme, betaine homocysteine methyltransferase (BHMT), also remethylates homocysteine to methionine using betaine as the methyl donor. Neither MS nor BHMT supports de novo methionine synthesis. Instead, their role is to recycle methionine consumed by AdoMet-dependent methylation reactions via the methionine cycle. Furthermore, AdoMet is utilized for polyamine synthesis, which generates methylthioadenosine as a byproduct. Methionine can also be recovered from methylthioadenosine via a salvage pathway. Therefore, under conditions of methionine conservation, activation of methionine recycling and salvage pathways provides a

mechanism for synthesizing AdoMet and polyamine without net methionine consumption. In normal cells, ~1% of the total AdoMet is diverted into polyamine synthesis, and this number can increase up to 100-fold in transformed cells (208, 243, 248, 275).

A second metabolic fate of homocysteine is its usage in the transsulfuration pathway that results in the irreversible exit of sulfur from the methionine cycle and into the synthesis of cysteine, H_2S, and other metabolites. The transsulfuration pathway comprises two enzymatic steps. In the first step, homocysteine is condensed with serine to form cystathionine in a reaction catalyzed by cystathionine β-synthase (CBS) followed by cleavage of cystathionine to α-ketobutyrate, ammonia, and cysteine in a reaction catalyzed by γ-cystathionase (CSE). In mammalian tissues, the transsulfuration pathway is irreversible. Hence, cysteine cannot be converted back to methionine, a capacity that is only present in lower organisms and renders methionine an essential amino acid for mammals. The transsulfuration pathway is present in the majority of tissues, including liver, kidney, pancreas, small intestine, and immune cells (macrophages, T cells, dendritic cells) (74, 87, 88). Although the presence of CSE in brain is debated (74, 119, 128), conversion of [^{35}S]-methionine into [^{35}S]-cysteine was observed in cultured human and murine neurons and astrocytes and in mouse brain slices (296), which provides evidence for an intact transsulfuration pathway in this organ. In contrast, the transsulfuration pathway does not appear to be intact in spleen, testes, heart, and skeletal muscle (74, 119).

Cysteine can be directed to several metabolic fates in addition to H_2S, and it is used for the synthesis of various other sulfur-containing metabolites, including GSH, coenzyme A (CoA), and taurine (Figure 1). Cysteine is a limiting substrate for fueling the synthesis of sulfur metabolites present at high intracellular concentrations, for example, GSH [up to 10 mM (164)] and taurine [ranging from 10 to 50 mM (114)]. The transsulfuration pathway is a significant source of cysteine in mammals, and its inhibition results in an ~50% decrease in GSH levels in cultured cells (26, 88, 183, 296) and in tissues (44, 59, 282). GSH is synthesized from cysteine in two ATP-dependent steps (164). In the first step, γ-glutamylcysteine is produced from glutamate and cysteine, catalyzed by γ-glutamate-cysteine ligase (GCL), which constitutes the rate-limiting step in GSH synthesis. In the second step, GSH is produced from γ-glutamylcysteine and glycine in a reaction catalyzed by GSH synthetase. The activity of GCL is subject to feedback inhibition by GSH that provides its redox sensitivity (228, 246) and is regulated by cysteine availability (169). Excess cysteine is committed to the oxidative degradation pathway by cysteine dioxygenase (CDO), which oxidizes cysteine to cysteinesulfinic acid, which in turn can be catabolized to pyruvate and sulfate via transamination or decarboxylated by cysteinesulfinate decarboxylase to produce hypotaurine. The latter is converted to taurine by a putative hypotaurine dehydrogenase (267). Hypotaurine can also be produced by oxidation of cysteamine, the end product of CoA degradation, in a reaction catalyzed by cysteamine dioxygenase (63).

Dietary Requirements

While the diet is the only source of methionine in mammals, cysteine can be produced from methionine via the transsulfuration pathway. The daily requirement of sulfur amino acids for

humans is ~13–15 mg/Kg (81, 152), with up to 89% of this is obtained as cystine, the oxidized disulfide form of cysteine (117, 151, 232). It is estimated that after digestion of food proteins, up to 20% of the methionine can be metabolized in gastrointestinal tissues (229). Excess dietary methionine has adverse physiological effects, although the mechanisms of its toxicity are not clear (19, 30, 81, 283, 304). In addition to being synthesized in the cysteine oxidation pathway, taurine is an abundant nonprotein amino acid that is obtained from the diet. Taurine is included in some energy drinks, although its usefulness in these drinks is questionable (48). In cats, which cannot synthesize taurine, it is an essential nutrient (325). The highest taurine concentrations are found in liver, muscle, and retina (114). The physiological role of taurine is not well understood, and functions ranging from an osmolyte (114, 181, 284), a neuromodulator (9, 113), an immunomodulator (245), and an antioxidant for detoxification of hypochlorous acid in immune cells (114) have been considered. Taurine deficiency leads to several abnormalities including blindness in some animals, decreased capacity for physical exercise, and heart dysfunction (113, 269, 303, 325). Recently, roles for taurine in intracellular pH buffering and stabilization of mitochondrial function have been proposed (100). Most other sulfur metabolites, such as AdoMet, exist at low concentrations in biological materials, and dietary input is not considered to be a significant source of sulfur. AdoMet is sold as the nutraceutical “SAMe” and is used in the treatment of alcoholic liver disease (25, 220) and for neurological problems (191, 205, 236) such as depressive disorders and Alzheimer’s disease. The pharmacological activity of SAMe is likely to be associated with its role as a universal methyl donor rather than with its sulfur content.

Transport of Sulfur Metabolites

The cellular concentration and turnover of sulfur metabolites are impacted by transport systems that connect pools across cell membranes. Plasma methionine levels in mouse, rat, and human are in the range of 20–100 μM (78, 97, 122, 165, 290, 293). Cysteine and GSH are present as a mixture of their reduced and oxidized forms, with total levels being ~100–200 μM for cysteine and ~10–25 μM for GSH (165, 227, 290, 293). In fact, the cysteine/cystine and GSH/oxidized glutathione levels represent redox nodes that buffer the extracellular redox potential, which is dynamically maintained by intracellular redox metabolism (182, 316). Fundamental cellular properties such as progression through the cell cycle and cell-cell interactions in the immune and neuroimmune systems are correlated with changes in the extracellular redox potential (182, 316). The concentration of taurine is ~50–90 μM in human and ~200–400 μM in rodent plasma (78, 165, 179, 293). Plasma concentrations of other sulfur metabolites such as homocysteine, AdoMet, and cystathionine are in the low micromolar range or they are present in trace quantities (11, 57, 97). Circulation provides a route for the exchange of sulfur compounds between different tissues.

In mammalian cells, cysteine is transported by multiple transport systems including the ubiquitous sodium-dependent systems A and ASC and sodium-independent systems L and *asc* (203, 257, 298). These transport systems can provide rapid uptake or efflux of cysteine depending on cellular demand. For cystine, the major transport system is x_C^-, which provides sodium-independent uptake of cystine in exchange for glutamate (203, 298). Despite its significance in cell metabolism, methionine transport across mammalian cell

membranes is poorly studied. In hepatocytes, methionine transport is fast, reversible, and Na^+-independent and provides equilibration of methionine between the cytoplasm and extracellular compartment (136, 147, 244). The system L transporter, LAT3, is primarily responsible for methionine transport in hepatocytes (16, 147). There are major gaps in our knowledge with regard to methionine transport in other cells. Upregulation of amino acid transporters in response to amino acid deprivation is well known (46). For instance, the cystine-glutamate exchanger is upregulated in human hepatocarcinoma cells deprived of cysteine/cystine (157, 252). Taurine is actively transported into cells by a specific Na^+-dependent transporter, TAUT (98). Genetic disruption of the *taut* gene results in dramatically decreased tissue taurine levels (303).

Due to the unusual γ-peptide bond between glutamate and cysteine, GSH is not destroyed by intracellular peptidases. GSH turnover is provided by its transport to the extracellular compartment and its degradation by the successive action of γ-glutamyl transpeptidase and a dipeptidase associated with the exterior surface of the cell membrane. The products of GSH hydrolysis are the component amino acids glutamate (as 5-oxoproline), cysteine, and glycine (164). The trans-membrane GSH cycle is thus a source of cysteine for the extracellular compartment, which is available to other cells and also helps maintain the cysteine/cystine redox poise. Reduced and oxidized GSH as well as GSH conjugates are pumped out by multi-drug-resistance transporters, contribute to GSH turnover, and detoxify xenobiotics (17, 20, 35). Additionally, some members of the family of organic anion transporters are involved in GSH export (17, 20). Although the transport of GSH into cells has been reported in yeast (17), analogous transporters appear to be absent in most mammalian cells with the exception of the small intestine epithelial cells (115), immortalized mouse brain endothelial cells (133), renal basolateral membranes (153, 154), and a rat kidney cell line, NRK-52E (155).

Transmembrane transporters for homocysteine, if they exist, are not known. Hepatocytes can transport homocysteine from cells to the extracellular medium (261). Similarly, little is known about the transport of AdoMet across the plasma membrane, although its transport efficiency is expected to be low because the ratio of AdoMet in tissues to plasma is ~1000:1 (11, 57, 146). AdoMet import into cells is also inefficient, as indicated by transport studies using extracellular AdoMet (243).

The permeability coefficient estimated for H_2S in human erythrocyte membrane is >0.01 cm s^{-1}, which is rapid enough to establish equilibration across the membrane in a fraction of a second (123), suggesting that H_2S transport across cells might occur via diffusion. In contrast, the sulfide anion would require facilitated transport, and it is proposed that the anion exchanger AE1 in human erythrocyte membranes serves this function (123).

Regulation of Sulfur Metabolism by Diet

Metabolism of diet-derived nutrients occurs primarily in the liver, which plays a central role in regulating circulating levels of sulfur metabolites in blood (166). Excess dietary intake of methionine or cysteine/cystine has toxic side effects (19) and if sustained can reduce growth and promote brain lesions and retinal degeneration (19, 211, 270). In an average diet, ~50% of methionine taken up by hepatocytes is regenerated by the action of MS and BHMT,

constituting the methionine conservation mode in metabolism (146). The remaining ~50% of methionine is metabolized via the transsulfuration pathway, which constitutes the only metabolic sink for methionine in mammals. Excess dietary intake of methionine activates its disposal via a sharp (~10-fold) increase in the rate of its consumption and conversion to cysteine in the hepatic transsulfuration pathway (Figure 2*a*) (75, 146, 185, 222). Interestingly, stable blood methionine levels are maintained despite fluctuations in dietary intake, including prolonged starvation (4, 5, 165). The mechanism of this stabilization is not clear and probably includes a shift toward its increased conservation via the methionine cycle and enhanced methionine supply from protein degradation. Switching between the methionine conservation and methionine disposal mode is provided by AdoMet-dependent allosteric regulation (Figure 2*a*). Under conditions of methyl group sufficiency, CBS is activated by AdoMet to increase diversion of homocysteine to the transsulfuration pathway. In parallel, AdoMet inhibits the remethylation of homocysteine to methionine by MS indirectly by inhibiting methylenetetrahydrofolate reductase (MTHFR), which provides the methyl group donor 5-methyl tetrahydrofolate, required by MS. At high concentrations, AdoMet also activates methionine adenosyltransferase III to further increase the conversion of methionine to AdoMet. Therefore, AdoMet has multiple targets for regulation and functions as an allosteric regulator, allowing for a sharp metabolic transition from the methionine conservation mode at low-to-normal methionine supply to the methionine disposal mode when the methionine supply is in excess (146). In the methionine conservation mode, the rate at which methionine is used for AdoMet synthesis is controlled by methionine adenosyltransferase I to support cellular methyl transfer reactions (Figure 2*b*). The expression of enzymes involved in the methionine disposal mode increases under prolonged increase in dietary methionine supply (75, 234). Under these conditions, cysteine- and homocysteine-dependent H_2S synthesis is predicted to increase by substrate-level activation. However, mechanistic insights into how cysteine is directed to H_2S synthesis in the normal or low metabolic mode are lacking.

Excess dietary cysteine is actively catabolized via desulfuration or oxidative degradation by CDO (267), which is highly expressed in liver (231, 267). CDO expression increases dramatically in response to increased dietary intake of protein or sulfur-containing amino acids (60–62, 268). Deletion of the CDO gene in mice leads to a significant increase in serum and tissue cysteine levels (231) and is associated with increased postnatal mortality, growth abnormality, and connective tissue problems (288). Overexpression of CDO in HepG2 cells reduces intracellular cysteine levels (62). Turnover of the intracellular GSH pool, which serves as a cysteine reservoir as well as an antioxidant, can be adjusted to respond to cellular cysteine needs (164).

The transsulfuration pathway is also activated under oxidative stress conditions (88, 183, 296) and downregulated by antioxidants (294), and the resulting changes in cysteine production are mirrored in GSH synthesis (88, 183, 296). Redox regulation of methionine metabolism is most likely provided by the redox sensitivity of MS (41), methionine adenosyltransferase (MAT), and CBS (238, 274).

Cysteine represents a highly regulated second junction in sulfur metabolism that can be directed into alternate pathways. Cysteine is consumed in the synthesis of GSH and CoA.

Although the quantitative significance of the flux of cysteine into CoA synthesis has not been assessed, it is believed to be small in comparison to GSH, which consumes cysteine at a rate that is comparable to its consumption by protein synthesis (222, 265, 291). However, a twofold increase in plasma cysteine levels in CDO-knockout mice results in only a negligible increase in GSH levels (231). The cellular concentration of cysteine is kept low by the action of CDO, which is upregulated at the transcriptional and posttranscriptional levels when dietary cysteine input is high. In contrast, under conditions of cysteine scarcity, CDO is ubiquitinated and targeted for proteasomal degradation (267). Tissue expression levels of CDO coincide with expression levels of the downstream enzyme cysteinesulfinate decarboxylase (267).

In rat hepatocytes, cysteine is cleared oxidatively via CDO (~63%) and by transamination/desulfuration (~37%), leading to H_2S production (267) (Figure 1). Approximately 66% of cysteinesulfinic acid produced in the CDO reaction is converted to hypotaurine and taurine, and ~34% is catabolized to pyruvate and sulfate (267). When the dietary intake of cysteine or methionine increases, cysteine catabolism via the CDO-dependent pathway increases significantly, whereas the amount metabolized via the desulfuration branch is more or less unchanged (18, 267).

In normal rat hepatocyte suspensions, the average rates of production of taurine plus hypotaurine, sulfate, and GSH are 33, 46, and 110 pmol min^{-1} mg^{-1} protein, respectively (18). Assuming that ~10% of cell mass is protein, we estimate that these rates are 198, 276, and 660 μmol h^{-1} Kg^{-1} cells. From these numbers and the partitioning of cysteine metabolic fluxes presented above, we estimate that the rate of H_2S production is ~180 μmol h^{-1} Kg^{-1} cells, i.e., ~27% the rate of GSH synthesis. This value is within the same order of magnitude reported for the average rate of H_2S production (484 μmol h^{-1} Kg^{-1}) in mouse liver (292). The interesting insight that emerges from this semiquantitative metabolic flux analysis is that the rate of hepatic H_2S production is comparable to the rates of taurine and GSH syntheses and must be taken into account during consideration of total metabolic sulfur balance or turnover. A cellular rationale for maintaining low cysteine levels is to avoid increased H_2S production that can in turn affect varied physiological processes.

H_2S METABOLISM

Enzymology of H_2S Production

H_2S is produced from the sulfur-containing metabolites cysteine and homocysteine by the enzymes of the transsulfuration pathway, CBS and CSE (43, 254), and from 3-mercaptopyruvate catalyzed by mercaptopyruvate sulfurtransferase (MST) and thioredoxin (170, 250) (Figure 1). CBS catalyzes the first irreversible step in the transsulfuration pathway, condensing homocysteine and serine to form cystathionine, which is cleaved by the second enzyme, CSE, to give cysteine. In addition to these canonical reactions, CBS and CSE exhibit substrate promiscuity and catalyze various H_2S-generating reactions from cysteine and homocysteine (43, 254) (Table 1). H_2S production by CBS and CSE results in the formation of two novel sulfur metabolites, lanthionine and homolanthionine (43, 254). Homolanthionine is reportedly elevated in urine of homocystinuric individuals (212), making it a potentially useful marker for homocysteine-dependent H_2S production.

The MST/thioredoxin system generates H_2S from 3-mercaptopyruvate (250) produced by transamination of cysteine that is catalyzed by cysteine (or aspartate) aminotransferase (286) (Figure 1). An alternative pathway for 3-mercaptopyruvate production involves oxidative deamination of D-cysteine catalyzed by D-amino acid oxidase, an activity that is localized in peroxisomes (111, 249). Mercaptopyruvate is desulfurated by MST to generate pyruvate and an active site per-sulfide, which transfers the sulfane sulfur to an acceptor and releases H_2S in the presence of reductants (170, 251). Kinetic analyses indicate that thioredoxin is an efficient acceptor of the MST-bound persulfide and can generate H_2S in the presence of thioredoxin reductase and nicotinamide adenine dinucleotide phosphate (NADPH) (171, 315). Although CBS, CSE, and MST are efficient at catalyzing H_2S generation under maximal velocity conditions, their relative contributions to endogenous H_2S generation vary with the tissue and are determined by the concentrations of the respective substrate and the expression levels of the enzymes (128).

The transsulfuration pathway plays a central role in sulfur metabolism (Figure 1). Commitment of homocysteine to the transsulfuration pathway serves at least two important functions: (*a*) to reduce levels of methionine under conditions of excess and to reduce levels of the toxic metabolite homocysteine and (*b*) to synthesize cysteine, an important precursor of other metabolites and the limiting substrate for GSH synthesis. Cysteine is a reactive amino acid, and its cellular concentration is maintained in the 80–100 μM range in most tissues (290) except in kidney, where its concentration is ~1 mM (266).

Unlike the other gas-signaling molecules CO and NO, whose synthesis is known to be highly regulated, H_2S appears to be primarily generated by housekeeping enzymes, and little is understood about how it is regulated. Additionally, based on kinetic considerations, the enzymes involved in H_2S biogenesis appear to be better poised to utilize substrates in the canonical, i.e., non-H_2S-generating, reactions. For example, serine, with an ~7- to 8-fold higher cellular concentration and ~4-fold lower K_M value as compared to cysteine, would be the preferred substrate for CBS under physiological conditions. Similarly, the relative intracellular concentrations of aspartate [0.7 mM in liver and 4 mM in brain (293)] versus cysteine [80–100 μM (290)], together with their K_M values for cysteine/aspartate aminotransferase [1.6 mM and 22 mM for aspartate and cysteine, respectively (7)], suggest that aspartate would be used preferentially by cysteine/aspartate aminotransferase. These considerations raise the obvious question as to how these enzymes are diverted away from catalyzing the canonical reactions and toward H_2S generation. CBS and CSE are cytoplasmic enzymes, although their nuclear localization has been seen under certain conditions (6, 129). MST is localized both in the cytoplasm and in the mitochondrion, suggesting a specific role for this enzyme in mitochondrial H_2S-based energy production. In agreement with this, mitochondrial cysteine concentration is high, ~0.7–1 mM, which would favor cysteine transamination by cysteine/aspartate aminotransferase (285). The regulation of H_2S biogenesis is an area that clearly deserves further attention.

Enzymology of H_2S Oxidation

The rapid metabolic removal of H_2S involves its oxidation to thiosulfate and sulfate, which was established by early metabolic labeling studies (55). The main H_2S oxidation product

excreted in urine is sulfate (27), whereas thiosulfate is the major end product of the colonic epithelium, which is exposed to high concentrations of sulfide produced by intestinal bacteria (85). H_2S is oxidized via a mitochondrial pathway linked to ATP production (103, 217), making H_2S the first known inorganic substrate for the electron transfer chain in mammals (94). Bacterial H_2S oxidation pathways are relatively well characterized (reviewed in 184). In mammals, four enzymes—sulfide quinone oxidoreductase (SQR), persulfide dioxygenase or ethylmalonic encephalopathy 1 (ETHE1), a sulfurtransferase or rhodanese, and sulfite oxidase—convert H_2S to persulfide, sulfite, thiosulfate, and sulfate, respectively (103) (Figure 3). In the first step, H_2S is oxidized by SQR, a flavoprotein localized in the inner mitochondrial membrane, forming a protein-bound persulfide. Two electrons from the oxidation of H_2S are transferred via flavin adenine dinucleotide to ubiquinone and then to the electron transport chain (42). The SQR-bound persulfide is transferred to an acceptor such as GSH or sulfite, resulting in the generation of GSH persulfide (103) or thiosulfate (120), respectively. Although the physiological acceptor for the SQR reaction is not known, GSH persulfide serves as substrate in the next step catalyzed by ETHE1 (103, 279). Furthermore, increased GSH synthesis by administration of the cysteine precursor *N*-acetylcysteine improves H_2S clearance in patients with ethylmalonic encephalopathy caused by mutations in the *ethe1* gene (289), implicating GSH as the persulfide carrier in the sulfide oxidation pathway. ETHE1 contains a nonheme mononuclear iron and oxidizes the persulfide to sulfite (127).

Recently, an alternative mechanism has been proposed based on the observation that sulfite, the product of the ETHE1 reaction, is an efficient acceptor of the SQR-borne persulfide and is converted to thiosulfate (120). If thiosulfate is formed during the SQR reaction, an additional sulfurtransferase such as rhodanese would have to be invoked to transfer the sulfane sulfur from thiosulfate to GSH to feed into the ETHE1 reaction (Figure 3). The experimental evidence for this step is lacking, and clearly, further studies are required for a better understanding of the organization of the H_2S oxidation pathway. Sulfite is efficiently converted to thiosulfate by rhodanese in a reaction in which GSH persulfide functions as the sulfane sulfur donor (103). Thiosulfate can be converted to sulfate by the actions of thiosulfate reductase (40) and sulfite oxidase (49).

Recent studies using sensitive methods to measure tissue H_2S and kinetic studies on the rates of H_2S formation and clearance have allowed assessment of steady-state H_2S levels, which are in the 10–30 nM range in many tissues (84, 292, 309). Endogenous H_2S biogenesis in liver drains a significant pool of sulfur that is ~27% of the flux into GSH synthesis. However, in contrast to GSH that is maintained at 1–10 mM steady-state concentrations, steady-state H_2S levels are ~10^5-fold lower. These data provide compelling evidence for a high flux of sulfur through the sulfide oxidation pathway to account for its low tissue concentrations (292), and indeed, experimental evidence supports a high capacity for H_2S oxidation by tissues (23, 55).

Regulation of Tissue H_2S Levels and Turnover

Despite the increasing number of reports in recent years on the physiological effects of H_2S, the field suffers from a paucity of reliable methods to determine tissue H_2S concentrations.

The values reported for normal H_2S concentrations vary over several orders of magnitude, with values in the hundreds of micromolar range representing the upper limit (196, 197). Accurate determination of tissue H_2S concentrations is mired in technical difficulties and experimental artifacts. Sulfide can easily leach out from iron-sulfur clusters that are abundant, resulting in gross overestimations of the free sulfide pool (84, 160, 196, 258). Exposure of rats to 800 ppm H_2S in air (~36 μM) leads to 100% mortality within minutes (229). Assuming equilibration between air and blood H_2S levels provides an estimated upper limit of ~36 μM for lethal H_2S blood concentration. Recent studies using sensitive methods such as sulfur chemiluminescence detection coupled to gas chromatography report tissue H_2S concentrations in the low nanomolar range (84, 197, 292, 309).

Tissue rates of H_2S production at physiologically relevant substrate concentrations range from 30–500 μmol h^{-1} Kg^{-1} tissue (292). Thus, intracellular H_2S concentrations could reach lethal levels in <60 minutes unless efficient routes for H_2S degradation existed. Indeed, the experimental data on H_2S clearance rates by murine liver, kidney, and brain demonstrate that these tissues maintain low steady-state H_2S levels in the nanomolar range by efficiently oxidizing H_2S (292), findings that are in agreement with other reports of low H_2S levels in vivo (84, 160, 196, 258).

The intestinal mucosa is exposed to high concentrations of H_2S produced by the degradation of sulfur-containing organic compounds and by dissimilatory sulfate reduction by the resident microflora. It is estimated that the total intestinal H_2S production can reach up to 12 mmol per day, which is equivalent to 6.6 μmol h^{-1} Kg^{-1} body weight for a 75-Kg person (85). Hence intestinal H_2S production could result in lethal concentrations in ~5 hours. This toxicity problem is averted by efficient H_2S oxidation in situ by the host, i.e., in the cecal and right colonic mucosa, which exhibit sulfide oxidation activity that are ~4- and 20-fold higher than in liver and muscle, respectively (85). It is unclear how much of the intestinal H_2S reaches the blood and other organs. Intestinal H_2S can influence total body H_2S levels under pathological conditions such as ethylmalonic encephalopathy (198). Indeed, it was shown in a recent study on the *ethe1*$^{-/-}$ mouse model for ethylmalonic encephalopathy that treatment with metronidazole, an antibiotic effective against anaerobic bacteria, causes an increase in the median survival time from 27 to 49 days (289). These data are consistent with a significant contribution of intestinal H_2S to systemic H_2S levels. Interestingly, a similar shift in median survival time (from 27 to 54.5 days) was observed when the mice received the GSH precursor *N*-acetylcysteine. Treatment with a combination of metronidazole and *N*-acetylcysteine had an additive effect and increased median survival time to 71.5 days. Additionally, combination therapy with metronidazole and *N*-acetylcysteine caused a marked clinical improvement in five children with ethylmalonic encephalopathy (289).

Based on the preceding discussion, it is clear that the levels of H_2S can be modulated by its production and/or its oxidation. Low steady-state concentrations of H_2S combined with a high rate of its turnover implies that the H_2S level can spike transiently, allowing for passage of a signal. Although the H_2S-producing enzymes in the transsulfuration pathway, particularly CBS, are highly regulated (226), their pertinence to H_2S production is not

known. Much less is known about the reaction mechanisms and regulation of enzymes involved in H_2S oxidation (103, 120, 127).

One level at which H_2S generation could be regulated is by substrate and cofactor availability. Critical enzymes in the sulfur network are dependent on cofactors obtained from the diet, including vitamins B_6, B_{12}, and folic acid in addition to zinc (Figure 1). Hence, dietary input of cysteine and homocysteine and the precursor methionine in addition to zinc and the B vitamins can modulate H_2S production. In addition, oxidative stress modulates the transsulfuration pathway and is relevant to diseases such as diabetes (66, 241, 281, 300). Elevated homocysteine, as found in homocystinuric patients, can increase CSE-dependent endogenous H_2S production (43, 254). AdoMet, an allosteric activator of CBS, enhances CBS activity ~2-fold in both the canonical and H_2S-producing reactions (314). Testosterone (219, 295), glucocorticoids (223), epidermal growth factor, transforming growth factor-α, cyclic adenosine monophosphate (cAMP), dexamethasone (69), and insulin (223) also modulate CBS activity. Nitric oxide (NO) reportedly stimulates H_2S production in rat fetal membranes and in aorta (209, 328) and increases CSE mRNA expression in aortic smooth muscle cells (328). Since MST-dependent H_2S biogenesis requires coupling to a reductant (e.g., the NADPH/thioredoxin reductase/thioredoxin) system (315), H_2S production by MST is expected to be sensitive to the intracellular redox status. Inhibition of 3-mercaptopyruvate production by cysteine/aspartate aminotransferase by aspartate that competes with cysteine (7), or by the reaction products glutamate or oxaloacetate, can decrease H_2S production by MST.

Several pharmacological inhibitors of CBS and CSE are quite widely used to modulate H_2S production and include propargylglycine, β-cyanoalanine, aminoethoxyvinylglycine, aminooxyacetic acid, hydroxylamine, and trifluoroalanine (3, 15, 299, 307). With the exception of propargylglycine, which is a mechanism-based inhibitor of CSE, the remaining compounds are relatively nonspecific and target other pyridoxal 5′-phosphate (PLP) enzymes (15). Propargylglycine is widely used in cell culture (26, 88, 183, 296) and in vivo (44, 59, 282) experiments. However, at high concentrations, propargylglycine also inhibits alanine aminotransferase (K_i = 3.9 mM) (38) and the cysteine/aspartate aminotransferase (at 100 mM propargylglycine) (273). Thus, in principle, the effect of propargylglycine in cell culture could result from inhibition of H_2S production by MST in addition to CSE.

β-cyanoalanine is used to target CSE and is a reversible inhibitor (180, 214, 328). However, it also inhibits aspartate decarboxylase and alanine aminotransferase (12, 51). Aminoethoxyvinyl-glycine, widely used to inhibit PLP-dependent ethylene synthesis in plants (323), also inhibits purified human CSE (262) and the transsulfuration pathway in cultured human astrocytes (296). Aminooxyacetic acid, an inhibitor of aminotransferases (225), is used to target CBS in vitro (2, 56, 107) and in vivo (235) but also inhibits CSE (15). Hence, aminooxyacetic acid is likely to inhibit all three routes for H_2S synthesis in cells. Despite this nonspecificity, aminooxyacetic acid inhibited H_2S production significantly less than did propargylglycine in smooth muscle cells (107), which might be due to a combination of factors such as poorer availability and a weaker inhibition constant. Although hydroxylamine is used as a specific CBS inhibitor (2), it is also widely used as a reagent for releasing the cofactor from PLP-dependent enzymes in general. Trifluoroalanine

is an irreversible inhibitor of PLP-dependent enzymes, which catalyze β- or γ-elimination reactions including CBS and CSE (253, 254, 262). Although it is ~4-fold more selective for CBS than for CSE (15), it has off-target activities as well.

H_2S AS A SIGNALING MOLECULE

Protein Persulfidation

A growing number of reports suggest a role for protein persulfidation (or *S*-sulfhydration) in H_2S-based signaling (186). Persulfidation can occur by the direct nucleophilic attack of the sulfide anion on an oxidized cysteine (e.g., a sulfenic acid, *S*-nitrosyl, or disulfide) by a transfer of an existing persulfide or by the nucleophilic attack of a cysteine thiolate on oxidized forms of H_2S [e.g., hydrogen disulfide (H_2S_2) or polysulfides] (95, 126) (Figure 4). The unstable chemical nature of persulfides and the absence of known mechanisms for achieving target specificity raise obvious questions regarding the prevalence and physiological relevance of this modification in H_2S-based signaling. However, the functional consequences of persulfidation on the activities of some enzymes have been recognized for over four decades (34, 101, 167). Although protein sulfhydration has been reported to be as prevalent as phosphorylation, with 10–25% of liver proteins being persulfidated, these data need to be treated with caution because they were obtained using a modified biotin switch assay (186), which lacks the specificity to distinguish between persulfides and thiols (204). The development of persulfide-specific reagents combined with direct demonstration of the persulfide modification in proteins is needed to assess the prevalence and validate the existence of this protein modification.

Persulfidation of some proteins has been demonstrated by mass spectrometric analysis, including glyceraldehyde-3-phosphate dehydrogenase (GAPDH), in which persulfidation increases activity (186), and the p65 subunit of nuclear factor-κB (NF-κB), which increases its antiapoptotic function in response to tumor necrosis factor-α (TNFα) treatment by increasing DNA binding and the transcriptional activity of NF-κB (247). *S*-nitrosylation of the same cysteine residues in GAPDH (247) and the p65 subunit of NF-κB (135) have opposing functional consequences. Persulfidation of the active site cysteine in protein tyrosine phosphatase (PTP)-1B inhibits its activity, resulting in the accumulation of phosphorylated PERK (protein kinase RNA-like endoplasmic reticulum kinase) and an enhanced cellular response to endoplasmic reticulum (ER) stress (149). Persulfidation of ATP-sensitive potassium channels results in channel opening and hyperpolarization of endothelial smooth muscle cells, leading to vasodilation (187, 328).

Interaction of H_2S with *S*-Nitrosothiol

The interaction between NO and H_2S signaling pathways is increasingly invoked as a potential mechanism for the action of H_2S. NO and H_2S exhibit some synergistic physiological effects that indicate cross talk between the two signaling systems (50, 71, 107, 162, 218, 280). Hints about a potential molecular mechanism for the interaction between the H_2S and NO signaling pathways came with the identification of thionitrous acid [*S*-nitrosothiol (HSNO)] generated by the reaction of H_2S and NO donor compounds (Figure 5*a*) (308). H_2S treatment blunts the effect of NO donor compounds and inhibits the NO-

dependent increase in cyclic guanosine 3′,5′-monophosphate (cGMP) levels, indicating adduct formation between H_2S and NO (308). Formation of HSNO by the reaction of H_2S with *S*-nitrosothiols, e.g., *S*-nitrosoglutathione (GSNO), has been demonstrated (73). HSNO freely diffuses through membranes, facilitates transnitrosation, and, in principle, can generate NO+, NO•, NO−, and HNO, potentially eliciting diverse specificities and physiological functions (73). The formation of HNO in vivo has been demonstrated using a nitroxyl-sensitive fluorescence probe (73). Despite its known cardioprotective role (76, 83, 134, 168, 206), the physiological relevance of HNO as a signaling molecule has been debated. Formation of HNO by a direct displacement of GSNO by GSH has been proposed (Figure 5) (255, 311). In these studies, formation of hydroxylamine and nitrous oxide in the reaction mixture indicated HNO formation because nitrous oxide can be generated by decomposition of the HNO dimerization product, hyponitrous acid (14, 260, 311).

The properties of HSNO investigated by high-level ab initio calculations indicate that the SNO group exists in mixed electronic states, with a predominant RS^-/NO^+ resonance structure accounting for its overall reactivity (276, 277). Recent studies indicate that the electronic structure of *S*-nitrosothiol (RSNO) could be modulated by its specific interactions with residues in proteins (272). A nucleophilic attack on the sulfur atom in the ion pair by a thiolate would yield HNO. In analogy with this, a nucleophilic attack by the sulfide anion on the sulfur atom in the RS^+/NO^-resonance form could produce HNO and a persulfide, representing a mechanism for persulfide formation. In the case of HSNO and H_2S as reactants, the products would be HNO and HSSH. It has been proposed that the pharmacological effects of sodium nitroprusside [$Na_2Fe(CN)_5(NO)$], used intravenously as a potent vasodilator, is mediated in part by HNO (72, 321). HNO can also function as a nitrosylating agent (82), and modulation of voltage-dependent K^+ channels in the vascular system in rats has been reported (116). The effect of Angeli's salt, an HNO donor, on myocyte contractility mimics the effects of sodium nitroprusside plus H_2S treatment (321).

Electrophile Sulfhydration

Sulfhydration of various electrophiles, including 8-nitro-cGMP nitro derivatives and keto derivatives of unsaturated fatty acids and cyclopentenone prostaglandin, has been demonstrated recently (193) (Figure 5*b*) and provides an additional molecular mechanism for H_2S signaling. Electrophiles function as signaling molecules and primarily react with cysteine residues to initiate signaling (77, 237, 259, 287). Protein modification by electrophiles such as S-guanylation by nitro-cGMP and electrophilic lipid-protein interactions (242, 259) mediates various redox-dependent signaling pathways (237) such as cGMP-dependent NO signaling (36). Denitration and sulfhydration of nitrated cGMP by H_2S block inducible nitric oxide synthase (iNOS)-dependent guanylation of H-Ras, a modification that activates H-Ras to signal cell senescence in response to stress (240, 312). Protein S-guanylation levels were increased by CBS knockdown in various human cell lines in culture (193). Increased 8-SH-cGMP levels and loss of H-Ras activation were proposed to be responsible in part for the protective role of H_2S in myocardial infarction–associated heart failure (193). The functional consequences for sulfhydration of compounds such as cyclopentenone prostaglandin (15d-PGJ2), which mediates electrophilic lipid-protein interactions by specifically modifying cysteine residues (259) and suppresses angiogenesis

(45, 80), are not known. The interaction between the H_2S and NO signaling pathways might be more common and also more complex than previously thought, and H_2S could modulate NO-based signaling either by mediating transnitrosylation between target molecules or by competing with NO for the same reactive cysteines.

Interaction of H_2S with Metal Centers

Another mechanism for H_2S-dependent signaling can be realized through its interaction with metal centers in the active sites of proteins. Depending on the stereoelectronic characteristics of the center, H_2S can reduce or coordinate to the metal (Figure 6). In hemeproteins, high H_2S concentrations promote heme reduction in polar active sites, whereas heme H_2S complexes are stabilized in nonpolar active sites (215). Because sulfide has a stronger nucleophilic character compared to water, it can displace water that generally fills empty coordination sites at metal centers in proteins. H_2S binds to both heme a_3 and Cu_B in the binuclear center (104) and reversibly inhibits cytochrome c oxidase, leading to a decrease in the metabolic rates and inducing a state of suspended animation in animals (32). The inhibition constant for sulfide is 0.2 μM for purified cytochrome c oxidase (213) and ~20 μM for intact cells (159). In isolated mitochondria, the maximal rates of respiration and ATP production were achieved in the presence of 10 μM H_2S, but the rates decreased with increasing sulfide concentration owing to inhibition of cytochrome c oxidase (322). Decreased ROS generation is another consequence of cytochrome c oxidase inhibition by H_2S and is believed to account for the protective effects of H_2S donors in ischemia-reperfusion-associated cardiac injury. Invertebrates living in sulfide-rich habitats use different forms of hemoglobin to transport sulfide to symbiotic bacteria to protect themselves from H_2S toxicity (297). It is not known whether coordination or reduction of metal centers by H_2S is involved in H_2S metabolism and signaling. Reduction of methemoglobin by H_2S can potentially lead to oxidative clearance of H_2S (28). Under aerobic conditions, H_2S can react with methemoglobin to form sulfhemoglobin, which represents an alternative sink for H_2S (192).

PHYSIOLOGICAL EFFECTS OF H_2S

The Effects of H_2S in the Central Nervous System

A physiological effect of H_2S unrelated to its toxicity was first reported in the central nervoussystem (CNS) where it was found to activate *N*-methyl-D-aspartate (NMDA) receptor–mediated responses and to facilitate induction of hippocampal long-term potentiation in the 10- to 130-μM concentration range (2). At higher concentrations (320 and 640 μM), sodium hydrosulfide (NaHS) inhibited synaptic transmission. In fact, reversible effects of H_2S on neuronal transmembrane potentials and currents were observed in toxicological studies with H_2S concentrations ranging from 30 to 400 μM (145). Expression of the $GABA_B$ receptor subunits 1 and 2 is upregulated by H_2S, whereas expression of the $GABA_B$ receptor subunit 2 but not 1 is inhibited by hydroxylamine, a nonspecific inhibitor of H_2S biogenesis (99). H_2S can affect levels of epinephrine, norepinephrine, and serotonin in brain (150, 233, 256, 302).

H_2S increases intracellular Ca^{2+} in neurons, astrocytes, and microglia by increasing Ca^{2+} influx into the cytoplasm from extracellular and intracellular compartments (86, 158, 190, 320), which can affect the interaction between these cells. Activation of voltage-dependent Ca^{2+} channels or transient receptor potential channels by H_2S is believed to be the underlying mechanism for intracellular Ca^{2+} increase (137, 138). H_2S can act as a neuroprotectant in the CNS by increasing production of GSH and by suppressing oxidative stress in mitochondria (140, 141). An H_2S-induced increase in GSH production is achieved through multiple mechanisms, including increased cystine and cysteine transport and activation of GCL without a concomitant change in the enzyme levels (140, 141). Although antioxidant activity via direct interaction between H_2S and reactive oxygen species has been invoked (137, 138), this seems unlikely to be a quantitatively significant mechanism owing to the low concentrations of H_2S in comparison with other antioxidants (e.g., GSH). Intraperitoneal treatment of pregnant rats with NaHS protects the fetal brain from damage caused by ischemia reperfusion, and this correlated with enhanced GSH levels (140). Cysteine supplementation promotes proliferation and differentiation of neuronal stem cells to neurons and astroglia, an effect that was attenuated by knockdown of CBS expression using siRNA (301). The neuroprotectant effects of H_2S could also be due to its anti-inflammatory and antiapoptosis activities (109) and the stabilization of membrane potentials (110, 139).

H_2S Effects in the Cardiovascular System

The first report of the effects of H_2S on the cardiovascular system indicated that it induced smooth muscle relaxation in the portal vein and the thoracic aorta (at 0.03–1.0 mM concentration) and enhanced vasorelaxation by NO (107). The relaxation mechanism is associated with opening of ATP-sensitive K^+ channels in an ATP-independent manner (328). H_2S-induced vasorelaxation is partially attenuated by removal of the endothelium, by inhibition of NO synthase, or by blockade of Ca^{2+}-dependent K^+ channels, suggesting that H_2S might stimulate endothelial cells to release one or more factors to facilitate smooth muscle relaxation (327). However, H_2S pretreatment is also reported to induce vasoconstriction by scavenging endothelial NO (10) and inhibiting the vasorelaxant effect of the NO donor sodium nitroprusside (327). Furthermore, at NaHS concentrations ranging from 5 to 100 μM, a reversal of its vasodilatory effects caused by phenylephrine precontracted rat thoracic aorta rings was observed in a mechanism that is likely to involve cAMP and AMP cyclase (163). In fact, H_2S-induced vasoconstriction and vasodilation in addition to multiphasic behavior have been observed in multiple vessels from different organisms (199). It is likely that the response of blood vessels to H_2S depends on multiple factors including vessel type, species, observation time, and H_2S concentration. Another variable that influences the effects of H_2S on the smooth muscle of blood vessels is O_2, with low and high concentrations inducing vasorelaxation and contraction, respectively (144).

Mice in which the *CSE* gene is disrupted exhibit hypertension in comparison with wild-type animals (319), although in a separate study, CSE-knockout mice were reported to be normotensive (118). The relaxation effect of H_2S on smooth muscle cells has also been observed in guinea pig ileum (107) and in the human corpus cavernosum strips, where effects were in the 0.1 to 10 mM range (56). H_2S can inhibit proliferation and cause

apoptosis of vascular smooth muscle cells (317, 318) and stimulate proliferation of vascular endothelial cells (39, 207). In these studies, the most significant effects were observed at H_2S concentrations ranging from 10 to 500 μM.

H_2S exerts a cardioprotective effect, and injecting 2.8 μmol Kg^{-1} day^{-1} or 14.0 μmol Kg^{-1} day^{-1} NaHS into rats treated with isoproterenol, which induces infarct-like myocardial necrosis, improved hemodynamic parameters, decreased subendocardial necrosis, and reduced animal mortality (89). In an isolated rat heart model for ischemia reperfusion, the addition of 1 μM NaHS to the perfusion buffer significantly reduced myocardial damage, whereas 0.1 μM and, surprisingly, 10 μM NaHS had no effect (125). Pretreatment with ATP-sensitive potassium channel blockers glibenclamide and sodium 5-hydroxydecanoate abrogated the protective effect of 1 μM NaHS. In another study using isolated rat heart and cultured myocytes, protection against ischemia reper-fusion injury was also observed, albeit at significantly higher NaHS concentrations (10–100 μM) (31). In an in vivo mouse model for ischemia reperfusion injury, similar protective effects were seen using 0.1 to 6.4 μmol Kg^{-1} H_2S, and maximal protection was obtained with an H_2S dose of 0.64 μmol Kg^{-1} (67). Significant protection against ischemia reperfusion injury was also achieved by cardiac-specific overexpression of CSE (67).

Endoplasmic Reticulum Stress

The ER provides a specialized compartment for folding of proteins targeted for the cell surface or for secretion. These proteins are rich in disulfide bonds critical for stability and function and for redox-dependent signaling. The ER lumen provides an oxidizing environment required for the formation of disulfide bonds and harbors stringent quality control mechanisms for ensuring fidelity. Various conditions, such as infection, inflammation, increased synthesis of secreted proteins in response to pathological conditions, or conditions that jeopardize protein folding, lead to compromised ER function and induction of the ER stress response. Misfolded proteins in the ER lumen activate autophosphorylation of ER-resident kinases including PERK. The latter in turn phosphorylates and inhibits eukaryotic initiator factor 2α (eIF2α), resulting in the inhibition of general protein synthesis to provide a window for the reestablishment of cellular homeostasis. Sulfhydration of an active site cysteine in PTP1B, a phosphatase that dephosphorylates PERK, results in inactivation leading to accumulation of phosphorylated PERK and enhanced cellular response to ER stress (149). Sulfhydration of PTP1B in response to ER stress is dependent on CSE activity (149). In agreement with these results, ER stress results in ATF4 (activating transcription factor 4)-dependent increase in CSE expression. Mouse embryonic fibroblast cells lacking the *CSE* gene are susceptible to ER stress–induced cell death (58). These results suggest regulatory and protective roles for H_2S during ER stress. ER stress accompanies many diseases such as diabetes (108), and it is chronically activated in all major cell types involved in atherosclerotic lesions (108, 189), which suggests that an enhanced ER stress response induced by H_2S could be partly responsible for the beneficial effects of H_2S.

Roles of H_2S in Aging

Because of its multiple physiological effects, H_2S can influence aging and age-related diseases such as cardiovascular diseases, neurodegenerative diseases, diabetes, and cancer (326). The nematode *Caenorhabditis elegans*, when grown in the presence of 50 ppm H_2S, exhibits increased thermo-tolerance and a 70% increase in life span (172). H_2S-induced life span increase in *C. elegans* is associated with increased expression of several age-related, stress-response, and antioxidant genes and protects animals against paraquat toxicity (221). Deletion of the *sir-2.1* gene encoding an NAD-dependent deacetylase abolished H_2S effects on thermotolerance and life span (172). H_2S also increases life span of the short-lived *mev-1 C. elegans* mutant (221). Mutations in *mev-1* encoding a homolog of the succinate dehydrogenase complex subunit C lead to hypersensitivity of animals to oxygen and paraquat and cause premature aging. Similarly, an increased mortality rate due to deficiency of *mpst-1* (encoding an MST ortholog) is reversed by exposing worms to H_2S, which indicates the importance of MST in H_2S production in this organism (221).

In mice, exposure to low levels of H_2S induces a suspended animation–like state by reversible inhibition of cytochrome c oxidase accompanied by a lowering of the metabolic rate and body temperature (32). Additional evidence indicates the importance of sulfur metabolism to aging. For instance, a low-methionine diet increases life span in rats (201) and in mice (173) and improves metabolic parameters (173). In agreement with these results, in hepatocytes from the long-lived Snell dwarf mice, methionine transport is several-fold lower, producing a functional methionine deficiency (293). In contrast, humans chronically exposed to H_2S in industrial settings (for an average of 12.0 ± 8.5 years) apparently exhibit signs of accelerated aging, detected by a statistically significant increase in serum levels of p53 and interleukin 8 (1).

H_2S-RELATED DEFICIENCIES IN SULFUR METABOLISM AND DISEASE

Enzymes poised at the sulfur metabolic junctions determine the partitioning of intermediates between alternative pathways and are strictly regulated. Deficiencies of these junction enzymes have pleiotropic consequences that affect major organ systems, including the cardiovascular and neuronal systems. High plasma homocysteine is an independent risk factor for cardiovascular diseases (224) and can result from deficiencies in CBS, MS, and other enzymes involved directly or indirectly in supporting sulfur metabolism at the homocysteine junction. Mutations in CBS are the most common cause of homocystinuria, and more than 150 mutations have been identified (148). Mutations in the *CBS* gene affect the ocular, cardiovascular, skeletal, and central nervous systems (47, 174, 224). Genetic deficiencies as well as low dietary levels of B_{12} and B_6, the cofactors for MS and CBS, respectively, can result in increased homocysteine levels. Other factors that lead to homocystinuria include a functional deficiency in MS caused by mutations in its gene or in the auxiliary protein methionine synthase reductase required for its reactivation, or a deficiency of its B_{12} cofactor or folate substrate (96, 156, 305, 306, 310). The B_{12} cofactor is targeted to MS via a complex mechanism, and defects in the trafficking proteins also lead to homocystinuria (21, 22, 90). Patients with MS deficiency present with a range of clinical abnormalities, including megaloblastic anemia and various neurological complications, such

as developmental problems, mental retardation, seizures, cerebral atrophy, and ataxia (263, 305). A low B_{12} level during pregnancy is correlated with neural tube defects (142, 174). MTHFR catalyzes the formation of 5-methyltetrahydrofolate, the methyl group donor in the reaction catalyzed by MS (Figure 1). MTHFR deficiency affects cellular folate metabolism, and a common polymorphism, C677T, which results in an alanine-to-valine substitution, leads to a thermolabile enzyme that is correlated with increased plasma homocysteine levels (79, 132). The prevalence of this polymorphism varies in different populations and ethnic backgrounds and is 5–15% in the United States and Europe (68, 132). During the catalytic cycle of MS, the B_{12} cofactor is occasionally oxidized and must be reduced to restore enzyme activity. MS reductase, a flavoprotein, provides the electron for the reductive activation of MS, and several mutations associated with homocystinuria have been described in its gene (156, 200, 310). A common MS reductase polymorphism with an ~51% prevalence in the US population is the I22M variant (310), which is correlated with coronary artery disease (37) and Down syndrome (105) and activates MS with lower efficiency (200).

Although plasma H_2S levels have not been measured in patients with deficient CBS and MS activities, kinetic studies predict that CSE-dependent H_2S production would be elevated in homocystinuric patients (43). In fact, homolanthionine, a by-product of the CSE-catalyzed condensation of two moles of homocysteine to generate H_2S, was detected in the urine of homocystinuric but not normal individuals, which indicates that H_2S formation is indeed elevated (212). Elevated homocysteine owing to defects in the remethylation pathway is expected to shift its utilization to the transsulfuration pathway and consequently to result in elevated H_2S synthesis (43).

In mouse models, deletion of CSE results in diminished plasma H_2S levels (319). Perturbations in sulfur metabolism have been reported in diabetic patients (102, 106, 112, 230) as well as in animal models of diabetes. The activities of CBS and CSE as well as homocysteine level (121) and H_2S production (324) are increased in the liver and kidney of streptozotocin-induced diabetic rats, a model for type 1 diabetes. CSE expression and H_2S production are also higher in pancreatic β-cells in Zucker diabetic fatty rats, a model for type 2 diabetes, compared to Zucker diabetic lean rats (313). However, plasma H_2S levels are low in nonobese diabetic mice (33). Patients with coronary artery occlusion or multivessel lesions showed significantly lower plasma H_2S concentrations (124). Brain H_2S levels in patients with Alzheimer’s disease are reported to be severely decreased (70). In patients with Down syndrome, serum homocysteine, methionine, AdoMet, and AdoHcy levels are significantly decreased whereas DNA methylation is increased (216), consistent with the presence of an extra copy of CBS on chromosome 21. Interestingly, urine thiosulfate is twofold higher in patients with Down syndrome compared to controls (29), indicating increased H_2S metabolism (131). Neuronal damage resulting from elevated H_2S production has been proposed to contribute to mental retardation in these patients (130).

In macrophages, CSE expression is reportedly increased in response to LPS and cytokines, with a concomitant increase in H_2S production (194). Increased plasma H_2S concentration also accompanies LPS-induced inflammation in mice (161). Patients with septic shock also display increased plasma H_2S (93). LPS treatment has been reported to increase serum sulfite levels in rats (178). Higher serum sulfite levels have also been reported in patients

with pneumonia, and neutrophils obtained from these patients produced higher amounts of sulfite in vitro compared to cells obtained after recovery (177), indicating increased synthesis of H_2S during the acute phase of the disease. Mutations in MST lead to mercaptopyruvate cysteine disulfiduria, an inborn error of metabolism characterized by mental retardation and elevated urinary excretion of mercaptopyruvate cysteine disulfide (53, 54).

More than 20 mutations have been described in patients with ethylmalonic encephalopathy due to deficiencies in ETHE1 (175, 278). Ethylmalonic encephalopathy is an autosomal recessive disorder characterized by severe pathophysiological abnormalities affecting the brain, gastrointestinal tract, and peripheral vessels; it leads to death in the first decade of life (175). Patients with ethylmalonic encephalopathy show unusual vascular features, acrocyanosis, bleeding abnormality due to microscopic hematuria and hemoperitoneum, hemorrhage, and dilated tortuous retinal vessels. Neuropathological features of the disease are necrotic lesions in the basal ganglia and in the brain stem (8). Biochemically, these patients exhibit increased plasma levels of H_2S, thiosulfate, and ethylmalonic acid; C4 and C5 acylcarnitines; elevated urinary excretion of ethylmalonic acid; and lactic acidemia. Cytochrome c oxidase activity is lower in skeletal muscles, presumably owing to inhibition by elevated H_2S levels (279).

CONCLUSIONS AND FUTURE DIRECTIONS

Recent years have witnessed exciting progress in elucidating the beneficial physiological effects of H_2S and in discovering potential mechanisms of its signaling action, such as via HSNO. However, the understanding of H_2S biology is hampered by significant gaps in our knowledge of the regulation of endogenous steady-state tissue H_2S levels, the rapid modulation of H_2S for inducing signaling, and the molecular targets of H_2S. Development of reliable methods for the detection and quantitation of H_2S and its targets of action is urgently needed to clarify controversies on its physiological effects. Because of the volatility and high turnover rates of H_2S in tissues, methods to quantify H_2S as well as techniques for H_2S sample preparation are crucial for obtaining reliable data. Although various probes to visualize endogenous H_2S levels have been developed, the majority lack the required stringency in specificity for H_2S, a fast response time, and the ratiometric output needed for quantitative analyses in cell-based assays (197). The intimate connection between dietary inputs into sulfur metabolism, particularly B vitamins and the amino acids methionine and cysteine, emphasizes the potentially important role of nutrition in modulating sulfur homeostasis and H_2S-based signaling. Studies assessing the interaction between nutrition and H_2S-based signaling represent another important gap in knowledge that warrants filling.

Acknowledgments

This work was supported in part by grants from the National Institutes of Health (HL58984 to R.B.) and the American Heart Association (13SDG17070096 to O.K.).

Glossary

H_2S	hydrogen sulfide
GSH	glutathione
AdoMet	*S*-adenosylmethionine
AdoHcy	*S*-adenosylhomocysteine
MS	methionine synthase
BHMT	betaine homocysteine methyltransferase
CBS	cystathionine β-synthase
CSE	γ-cystathionase
CoA	coenzyme A
GCL	γ-glutamate-cysteine ligase
CDO	cysteine dioxygenase
MTHFR	methylenetetrahydro-folate reductase
MST	mercaptopyruvate sulfurtransferase
SQR	sulfide quinone oxidoreductase
ETHE1	persulfide dioxygenase or ethylmalonic encephalopathy 1
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
ER	endoplasmic reticulum

LITERATURE CITED

1. Abdrashitova AT, Panova TN, Belolapenko IA. Changes in ageing pace and major immune parameters among individuals with long exposure to hydrogen sulfide. Med Tr Prom Ekol. 2011; 7:10–16. [PubMed: 21938919]
2. Abe K, Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. J Neurosci. 1996; 16:1066–71. [PubMed: 8558235]
3. Abeles RH, Walsh C. Acetylenic enzyme inactivators. Inactivation of γ-cystathionase. J Am Chem Soc. 1973; 95:6124–25. [PubMed: 4733835]
4. Achouri Y, Robbi M, Van Schaftingen E. Role of cysteine in the dietary control of the expression of 3-phosphoglycerate dehydrogenase in rat liver. Biochem J. 1999; 344(Part 1):15–21. [PubMed: 10548528]
5. Adibi SA. Interrelationships between level of amino acids in plasma and tissues during starvation. Am J Physiol. 1971; 221:829–38. [PubMed: 5570339]
6. Agrawal N, Banerjee R. Human polycomb 2 protein is a SUMO E3 ligase and alleviates substrate-induced inhibition of cystathionine beta-synthase sumoylation. PLoS ONE. 2008; 3:e4032. [PubMed: 19107218]
7. Akagi R. Purification and characterization of cysteine aminotransferase from rat liver cytosol. Acta Med Okayama. 1982; 36:187–97. [PubMed: 7113743]
8. Al-Ajmi MO, Kutty SK. A case report—ethyl malonic aciduria encephalopathy with respiratory failure and nephrotic syndrome rare presentation. Middle East J Fam Med. 2005; 3:29–32.
9. Albrecht J, Schousboe A. Taurine interaction with neurotransmitter receptors in the CNS: an update. Neurochem Res. 2005; 30:1615–21. [PubMed: 16362781]

10. Ali MY, Ping CY, Mok YY, Ling L, Whiteman M, et al. Regulation of vascular nitric oxide in vitro and in vivo; a new role for endogenous hydrogen sulphide? Br J Pharmacol. 2006; 149:625–34. [PubMed: 17016507]
11. Alsayed R, Al Quobaili F, Srour S, Geisel J, Obeid R. Elevated dimethylglycine in blood of children with congenital heart defects and their mothers. Metabolism. 2013; 62:1074–80. [PubMed: 23481916]
12. Alston TA, Porter DJ, Mela L, Bright HJ. Inactivation of alanine aminotransferase by the neurotoxin beta-cyano-L-alanine. Biochem Biophys Res Commun. 1980; 92:299–304. [PubMed: 7356461]
13. Arndt C, Gaill F, Felbeck H. Anaerobic sulfur metabolism in thiotrophic symbioses. J Exp Biol. 2001; 204:741–50. [PubMed: 11171356]
14. Arnelle DR, Stamler JS. NO+, NO, and NO- donation by *S*-nitrosothiols: implications for regulation of physiological functions by *S*-nitrosylation and acceleration of disulfide formation. Arch Biochem Biophys. 1995; 318:279–85. [PubMed: 7733655]
15. Asimakopoulou A, Panopoulos P, Chasapis CT, Coletta C, Zhou Z, et al. Selectivity of commonly used pharmacological inhibitors for cystathionine beta synthase (CBS) and cystathionine gamma lyase (CSE). Br J Pharmacol. 2013; 169:922–32. [PubMed: 23488457]
16. Babu E, Kanai Y, Chairoungdua A, Kim DK, Iribe Y, et al. Identification of a novel system L amino acid transporter structurally distinct from heterodimeric amino acid transporters. J Biol Chem. 2003; 278:43838–45. [PubMed: 12930836]
17. Bachhawat AK, Thakur A, Kaur J, Zulkifli M. Glutathione transporters. Biochim Biophys Acta. 2013; 1830:3154–64. [PubMed: 23206830]
18. Bagley PJ, Stipanuk MH. Rats fed a low protein diet supplemented with sulfur amino acids have increased cysteine dioxygenase activity and increased taurine production in hepatocytes. J Nutr. 1995; 125:933–40. [PubMed: 7722697]
19. Baker DH. Comparative species utilization and toxicity of sulfur amino acids. J Nutr. 2006; 136:1670–75S.
20. Ballatori N, Hammond CL, Cunningham JB, Krance SM, Marchan R. Molecular mechanisms of reduced glutathione transport: role of the MRP/CFTR/ABCC and OATP/SLC21A families of membrane proteins. Toxicol Appl Pharmacol. 2005; 204:238–55. [PubMed: 15845416]
21. Banerjee R. B_{12} trafficking in mammals: a case for coenzyme escort service. ACS Chem Biol. 2006; 1:149–59. [PubMed: 17163662]
22. Banerjee R, Gherasim C, Padovani D. The tinker, tailor, soldier in intracellular B_{12} trafficking. Curr Opin Chem Biol. 2009; 13:484–91. [PubMed: 19665918]
23. Bartholomew TC, Powell GM, Dodgson KS, Curtis CG. Oxidation of sodium sulphide by rat liver, lungs and kidney. Biochem Pharmacol. 1980; 29:2431–37. [PubMed: 7426049]
24. Barton LL, Fauque GD. Biochemistry, physiology and biotechnology of sulfate-reducing bacteria. Adv Appl Microbiol. 2009; 68:41–98. [PubMed: 19426853]
25. Barve A, Khan R, Marsano L, Ravindra KV, McClain C. Treatment of alcoholic liver disease. Ann Hepatol. 2008; 7:5–15. [PubMed: 18376362]
26. Beatty PW, Reed DJ. Involvement of the cystathionine pathway in the biosynthesis of glutathione by isolated rat hepatocytes. Arch Biochem Biophys. 1980; 204:80–87. [PubMed: 7425648]
27. Beauchamp RO Jr, Bus JS, Popp JA, Boreiko CJ, Andjelkovich DA. A critical review of the literature on hydrogen sulfide toxicity. Crit Rev Toxicol. 1984; 13:25–97. [PubMed: 6378532]
28. Beck JF, Bradbury CM, Connors AJ, Donini JC. Nitrite as antidote for acute hydrogen sulfide intoxication? Am Ind Hyg Assoc J. 1981; 42:805–9. [PubMed: 7315740]
29. Belardinelli MC, Chabli A, Chadefaux-Vekemans B, Kamoun P. Urinary sulfur compounds in Down syndrome. Clin Chem. 2001; 47:1500–1. [PubMed: 11468253]
30. Benevenga NJ, Steele RD. Adverse effects of excessive consumption of amino acids. Annu Rev Nutr. 1984; 4:157–81. [PubMed: 6235826]
31. Bian JS, Yong QC, Pan TT, Feng ZN, Ali MY, et al. Role of hydrogen sulfide in the cardioprotection caused by ischemic preconditioning in the rat heart and cardiac myocytes. J Pharmacol Exp Ther. 2006; 316:670–78. [PubMed: 16204473]

32. Blackstone E, Morrison M, Roth MB. H_2S induces a suspended animation-like state in mice. Science. 2005; 308:518. [PubMed: 15845845]
33. Brancaleone V, Roviezzo F, Vellecco V, De Gruttola L, Bucci M, Cirino G. Biosynthesis of H_2S is impaired in non-obese diabetic (NOD) mice. Br J Pharmacol. 2008; 155:673–80. [PubMed: 18641671]
34. Branzoli U, Massey V. Evidence for an active site persulfide residue in rabbit liver aldehyde oxidase. J Biol Chem. 1974; 249:4346–49. [PubMed: 4276457]
35. Brechbuhl HM, Gould N, Kachadourian R, Riekhof WR, Voelker DR, Day BJ. Glutathione transport is a unique function of the ATP-binding cassette protein ABCG2. J Biol Chem. 2010; 285:16582–87. [PubMed: 20332504]
36. Bredt DS, Hwang PM, Snyder SH. Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature. 1990; 347:768–70. [PubMed: 1700301]
37. Brown CA, McKinney KQ, Kaufman JS, Gravel RA, Rozen R. A common polymorphism in methionine synthase reductase increases risk of premature coronary artery disease. J Cardiovasc Risk. 2000; 7:197–200. [PubMed: 11006889]
38. Burnett G, Marcotte P, Walsh C. Mechanism-based inactivation of pig heart L-alanine transaminase by L-propargylglycine. Half-site reactivity. J Biol Chem. 1980; 255:3487–91. [PubMed: 7364752]
39. Cai WJ, Wang MJ, Moore PK, Jin HM, Yao T, Zhu YC. The novel proangiogenic effect of hydrogen sulfide is dependent on Akt phosphorylation. Cardiovasc Res. 2007; 76:29–40. [PubMed: 17631873]
40. Chauncey TR, Uhteg LC, Westley J. Thiosulfate reductase. Methods Enzymol. 1987; 143:350–4. [PubMed: 3309558]
41. Chen, Z.; Banerjee, R. Characterization of the Human and Porcine Methionine Synthases and Their Redox Partners. New York: Kluwer; 1996.
42. Cherney MM, Zhang Y, Solomonson M, Weiner JH, James MN. Crystal structure of sulfide:quinone oxidoreductase from *Acidithiobacillus ferrooxidans*: insights into sulfidotrophic respiration and detoxification. J Mol Biol. 2010; 398:292–305. [PubMed: 20303979]
43. Chiku T, Padovani D, Zhu W, Singh S, Vitvitsky V, Banerjee R. H_2S biogenesis by human cystathionine gamma-lyase leads to the novel sulfur metabolites lanthionine and homolanthionine and is responsive to the grade of hyperhomocysteinemia. J Biol Chem. 2009; 284:11601–12. [PubMed: 19261609]
44. Cho ES, Hovanec-Brown J, Tomanek RJ, Stegink LD. Propargylglycine infusion effects on tissue glutathione levels, plasma amino acid concentrations and tissue morphology in parenterally-fed growing rats. J Nutr. 1991; 121:785–94. [PubMed: 2033464]
45. Cho WH, Choi CH, Park JY, Kang SK, Kim YK. 15-deoxy-(Δ12,14)-prostaglandin J2 (15d-PGJ2) induces cell death through caspase-independent mechanism in A172 human glioma cells. Neurochem Res. 2006; 31:1247–54. [PubMed: 17006759]
46. Christie GR, Hyde R, Hundal HS. Regulation of amino acid transporters by amino acid availability. Curr Opin Clin Nutr Metab Care. 2001; 4:425–31. [PubMed: 11568505]
47. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol. 1998; 55:1449–55. [PubMed: 9823829]
48. Clauson KA, Shields KM, McQueen CE, Persad N. Safety issues associated with commercially available energy drinks. J Am Pharm Assoc. 2008; 48:e55–63. quiz e64–67.
49. Cohen HJ, Betcher-Lange S, Kessler DL, Rajagopalan KV. Hepatic sulfite oxidase. Congruency in mitochondria of prosthetic groups and activity. J Biol Chem. 1972; 247:7759–66. [PubMed: 4344230]
50. Coletta C, Papapetropoulos A, Erdelyi K, Olah G, Modis K, et al. Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation. Proc Natl Acad Sci USA. 2012; 109:9161–66. [PubMed: 22570497]
51. Cornell NW, Zuurendonk PF, Kerich MJ, Straight CB. Selective inhibition of alanine aminotransferase and aspartate aminotransferase in rat hepatocytes. Biochem J. 1984; 220:707–16. [PubMed: 6466297]

52. Costantino M, Filippelli A, Quenau P, Nicolas JP, Coiro V. Sulphur mineral water and SPA therapy in osteoarthritis. Therapie. 2012; 67:43–48. [PubMed: 22487501]
53. Crawhall JC, Parker R, Sneddon W, Young EP. Beta-mercaptolactate-cysteine disulfide in the urine of a mentally retarded patient. Am J Dis Child. 1969; 117:71–82. [PubMed: 5812433]
54. Crawhall JC, Parker R, Sneddon W, Young EP, Ampola MG, et al. Beta-mercaptolactate-cysteine disulfide: analog of cystine in the urine of a mentally retarded patient. Science. 1968; 160:419–20. [PubMed: 5644041]
55. Curtis CG, Bartholomew TC, Rose FA, Dodgson KS. Detoxication of sodium 35 S-sulphide in the rat. Biochem Pharmacol. 1972; 21:2313–21. [PubMed: 4647068]
56. d'Emmanuele di Villa Bianca R, Sorrentino R, Maffia P, Mirone V, Imbimbo C, et al. Hydrogen sulfide as a mediator of human corpus cavernosum smooth-muscle relaxation. Proc Natl Acad Sci USA. 2009; 106:4513–18. [PubMed: 19255435]
57. Dayal S, Bottiglieri T, Arning E, Maeda N, Malinow MR, et al. Endothelial dysfunction and elevation of *S*-adenosylhomocysteine in cystathionine beta-synthase-deficient mice. Circ Res. 2001; 88:1203–9. [PubMed: 11397788]
58. Dickhout JG, Carlisle RE, Jerome DE, Mohammed-Ali Z, Jiang H, et al. Integrated stress response modulates cellular redox state via induction of cystathionine gamma-lyase: cross-talk between integrated stress response and thiol metabolism. J Biol Chem. 2012; 287:7603–14. [PubMed: 22215680]
59. Diwakar L, Ravindranath V. Inhibition of cystathionine-gamma-lyase leads to loss of glutathione and aggravation of mitochondrial dysfunction mediated by excitatory amino acid in the CNS. Neurochem Int. 2007; 50:418–26. [PubMed: 17095121]
60. Dominy JE Jr, Hirschberger LL, Coloso RM, Stipanuk MH. Regulation of cysteine dioxygenase degradation is mediated by intracellular cysteine levels and the ubiquitin-26 S proteasome system in the living rat. Biochem J. 2006; 394:267–73. [PubMed: 16262602]
61. Dominy JE Jr, Hwang J, Guo S, Hirschberger LL, Zhang S, Stipanuk MH. Synthesis of amino acid cofactor in cysteine dioxygenase is regulated by substrate and represents a novel post-translational regulation of activity. J Biol Chem. 2008; 283:12188–201. [PubMed: 18308719]
62. Dominy JE Jr, Hwang J, Stipanuk MH. Overexpression of cysteine dioxygenase reduces intracellular cysteine and glutathione pools in HepG2/C3A cells. Am J Physiol Endocrinol Metab. 2007; 293:E62–69. [PubMed: 17327371]
63. Dominy JE Jr, Simmons CR, Hirschberger LL, Hwang J, Coloso RM, Stipanuk MH. Discovery and characterization of a second mammalian thiol dioxygenase, cysteamine dioxygenase. J Biol Chem. 2007; 282:25189–98. [PubMed: 17581819]
64. Dubilier N, Mulders C, Ferdelman T, de Beer D, Pernthaler A, et al. Endosymbiotic sulphate-reducing and sulphide-oxidizing bacteria in an oligochaete worm. Nature. 2001; 411:298–302. [PubMed: 11357130]
65. Duperron S, Guezi H, Gaudron SM, Pop Ristova P, Wenzhofer F, Boetius A. Relative abundances of methane- and sulphur-oxidising symbionts in the gills of a cold seep mussel and link to their potential energy sources. Geobiology. 2011; 9:481–91. [PubMed: 21978364]
66. Ebesunun MO, Obajobi EO. Elevated plasma homocysteine in type 2 diabetes mellitus: a risk factor for cardiovascular diseases. Pan Afr Med J. 2012; 12:48. [PubMed: 22937188]
67. Elrod JW, Calvert JW, Morrison J, Doeller JE, Kraus DW, et al. Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. Proc Natl Acad Sci USA. 2007; 104:15560–65. [PubMed: 17878306]
68. Engbersen AM, Franken DG, Boers GH, Stevens EM, Trijbels FJ, Blom HJ. Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. Am J Hum Genet. 1995; 56:142–50. [PubMed: 7825569]
69. Enokido Y, Suzuki E, Iwasawa K, Namekata K, Okazawa H, Kimura H. Cystathionine β-synthase, a key enzyme for homocysteine metabolism, is preferentially expressed in the radial glia/astrocyte lineage of developing mouse CNS. FASEB J. 2005; 19:1854–56. [PubMed: 16160063]
70. Eto K, Asada T, Arima K, Makifuchi T, Kimura H. Brain hydrogen sulfide is severely decreased in Alzheimer's disease. Biochem Biophys Res Commun. 2002; 293:1485–88. [PubMed: 12054683]

71. Fago A, Jensen FB, Tota B, Feelisch M, Olson KR, et al. Integrating nitric oxide, nitrite and hydrogen sulfide signaling in the physiological adaptations to hypoxia: a comparative approach. Comp Biochem Physiol A Mol Integr Physiol. 2012; 162:1–6. [PubMed: 22314020]
72. Filipovic MR, Eberhardt M, Prokopovic V, Mijuskovic A, Orescanin-Dusic Z, et al. Beyond H_2S and NO interplay: hydrogen sulfide and nitroprusside react directly to give nitroxyl (HNO). A new pharmacological source of HNO. J Med Chem. 2013; 56:1499–508. [PubMed: 23418783]
73. Filipovic MR, Miljkovic J, Nauser T, Royzen M, Klos K, et al. Chemical characterization of the smallest *S*-nitrosothiol, HSNO; cellular cross-talk of H_2S and *S*-nitrosothiols. J Am Chem Soc. 2012; 134:12016–27. [PubMed: 22741609]
74. Finkelstein JD. Pathways and regulation of homocysteine metabolism in mammals. Semin Thromb Hemost. 2000; 26:219–25. [PubMed: 11011839]
75. Finkelstein JD, Martin JJ. Methionine metabolism in mammals. Adaptation to methionine excess. J Biol Chem. 1986; 261:1582–87. [PubMed: 3080429]
76. Flores-Santana W, Salmon DJ, Donzelli S, Switzer CH, Basudhar D, et al. The specificity of nitroxyl chemistry is unique among nitrogen oxides in biological systems. Antioxid Redox Signal. 2011; 14:1659–74. [PubMed: 21235346]
77. Forman HJ, Fukuto JM, Torres M. Redox signaling: Thiol chemistry defines which reactive oxygen and nitrogen species can act as second messengers. Am J Physiol Cell Physiol. 2004; 287:C246–56. [PubMed: 15238356]
78. Forslund AH, Hambraeus L, van Beurden H, Holmback U, El-Khoury AE, et al. Inverse relationship between protein intake and plasma free amino acids in healthy men at physical exercise. Am J Physiol Endocrinol Metab. 2000; 278:E857–67. [PubMed: 10780942]
79. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995; 10:111–13. [PubMed: 7647779]
80. Fu YG, Sung JJ, Wu KC, Bai AH, Chan MC, et al. Inhibition of gastric cancer cells associated angiogenesis by 15d-prostaglandin J2 through the downregulation of angiopoietin-1. Cancer Lett. 2006; 243:246–54. [PubMed: 16412567]
81. Fukagawa NK, Galbraith RA. Advancing age and other factors influencing the balance between amino acid requirements and toxicity. J Nutr. 2004; 134:1569–74S.
82. Fukuto JM, Bartberger MD, Dutton AS, Paolocci N, Wink DA, Houk KN. The physiological chemistry and biological activity of nitroxyl (HNO): the neglected, misunderstood, and enigmatic nitrogen oxide. Chem Res Toxicol. 2005; 18:790–801. [PubMed: 15892572]
83. Fukuto JM, Chiang K, Hszieh R, Wong P, Chaudhuri G. The pharmacological activity of nitroxyl: a potent vasodilator with activity similar to nitric oxide and/or endothelium-derived relaxing factor. J Pharmacol Exp Ther. 1992; 263:546–51. [PubMed: 1331403]
84. Furne J, Saeed A, Levitt MD. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. Am J Physiol Regul Integr Comp Physiol. 2008; 295:R1479–85. [PubMed: 18799635]
85. Furne J, Springfield J, Koenig T, DeMaster E, Levitt MD. Oxidation of hydrogen sulfide and methanethiol to thiosulfate by rat tissues: a specialized function of the colonic mucosa. Biochem Pharmacol. 2001; 62:255–59. [PubMed: 11389886]
86. Garcia-Bereguiain MA, Samhan-Arias AK, Martin-Romero FJ, Gutierrez-Merino C. Hydrogen sulfide raises cytosolic calcium in neurons through activation of L-type Ca^{2+} channels. Antioxid Redox Signal. 2008; 10:31–42. [PubMed: 17956188]
87. Garg S, Vitvitsky V, Gendelman HE, Banerjee R. Monocyte differentiation, activation, and mycobacterial killing are linked to transsulfuration-dependent redox metabolism. J Biol Chem. 2006; 281:38712–20. [PubMed: 17046819]
88. Garg SK, Yan Z, Vitvitsky V, Banerjee R. Differential dependence on cysteine from transsulfuration versus transport during T cell activation. Antioxid Redox Signal. 2011; 15:39–47. [PubMed: 20673163]
89. Geng B, Chang L, Pan C, Qi Y, Zhao J, et al. Endogenous hydrogen sulfide regulation of myocardial injury induced by isoproterenol. Biochem Biophys Res Commun. 2004; 318:756–63. [PubMed: 15144903]

90. Gherasim C, Lofgren M, Banerjee R. Navigating the B_{12} road: assimilation, delivery and disorders of cobalamin. J Biol Chem. 2013; 288:13186–93. [PubMed: 23539619]
91. Ghosh W, Dam B. Biochemistry and molecular biology of lithotrophic sulfur oxidation by taxonomically and ecologically diverse bacteria and archaea. FEMS Microbiol Rev. 2009; 33:999–1043. [PubMed: 19645821]
92. Girguis PR, Childress JJ. Metabolite uptake, stoichiometry and chemoautotrophic function of the hydrothermal vent tubeworm *Riftia pachyptila*: responses to environmental variations in substrate concentrations and temperature. J Exp Biol. 2006; 209:3516–28. [PubMed: 16943492]
93. Goslar T, Mars T, Podbregar M. Total plasma sulfide as a marker of shock severity in nonsurgical adult patients. Shock. 2011; 36:350–55. [PubMed: 21897337]
94. Goubern M, Andriamihaja M, Nubel T, Blachier F, Bouillaud F. Sulfide, the first inorganic substrate for human cells. FASEB J. 2007; 21:1699–706. [PubMed: 17314140]
95. Greiner R, Palinkas Z, Basell K, Becher D, Antelmann H, et al. Polysulfides link H_2S to protein thiol oxidation. Antioxid Redox Signal. 2013; 19:1749–65. [PubMed: 23646934]
96. Gulati S, Baker P, Li YN, Fowler B, Kruger W, et al. Defects in human methionine synthase in cblG patients. Hum Mol Genet. 1996; 5:1859–65. [PubMed: 8968736]
97. Guttormsen AB, Solheim E, Refsum H. Variation in plasma cystathionine and its relation to changes in plasma concentrations of homocysteine and methionine in healthy subjects during a 24-h observation period. Am J Clin Nutr. 2004; 79:76–79. [PubMed: 14684400]
98. Han X, Patters AB, Jones DP, Zelikovic I, Chesney RW. The taurine transporter: mechanisms of regulation. Acta Physiol. 2006; 187:61–73.
99. Han Y, Qin J, Chang X, Yang Z, Tang X, Du J. Hydrogen sulfide may improve the hippocampal damage induced by recurrent febrile seizures in rats. Biochem Biophys Res Commun. 2005; 327:431–36. [PubMed: 15629133]
100. Hansen SH, Grunnet N. Taurine, glutathione and bioenergetics. Adv Exp Med Biol. 2013; 776:3–12. [PubMed: 23392865]
101. Hargrove JL. Persulfide generated from L-cysteine inactivates tyrosine aminotransferase. Requirement for a protein with cysteine oxidase activity and gamma-cystathionase. J Biol Chem. 1988; 263:17262–69. [PubMed: 2903161]
102. Herrmann W, Schorr H, Obeid R, Makowski J, Fowler B, Kuhlmann MK. Disturbed homocysteine and methionine cycle intermediates *S*-adenosylhomocysteine and *S*-adenosylmethionine are related to degree of renal insufficiency in type 2 diabetes. Clin Chem. 2005; 51:891–97. [PubMed: 15774574]
103. Hildebrandt TM, Grieshaber MK. Three enzymatic activities catalyze the oxidation of sulfide to thiosulfate in mammalian and invertebrate mitochondria. FEBS J. 2008; 275:3352–61. [PubMed: 18494801]
104. Hill BC, Woon TC, Nicholls P, Peterson J, Greenwood C, Thomson AJ. Interactions of sulphide and other ligands with cytochrome c oxidase. An electron-paramagnetic-resonance study. Biochem J. 1984; 224:591–600. [PubMed: 6097224]
105. Hobbs CA, Sherman SL, Yi P, Hopkins SE, Torfs CP, et al. Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome. Am J Hum Genet. 2000; 67:623–30. [PubMed: 10930360]
106. Hoogeveen EK, Kostense PJ, Beks PJ, Mackaay AJ, Jakobs C, et al. Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non-insulin-dependent diabetes mellitus: a population-based study. Arterioscler Thromb Vasc Biol. 1998; 18:133–38. [PubMed: 9445267]
107. Hosoki R, Matsuki N, Kimura H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. Biochem Biophys Res Commun. 1997; 237:527–31. [PubMed: 9299397]
108. Hotamisligil GS. Endoplasmic reticulum stress and atherosclerosis. Nat Med. 2010; 16:396–99. [PubMed: 20376052]
109. Hu LF, Lu M, Hon Wong PT, Bian JS. Hydrogen sulfide: neurophysiology and neuropathology. Antioxid Redox Signal. 2011; 15:405–19. [PubMed: 20812864]

110. Hu LF, Lu M, Wu ZY, Wong PT-H, Bian JS. Hydrogen sulfide inhibits rotenone-induced apoptosis via preservation of mitochondrial function. Mol Pharmacol. 2009; 75:27–34. [PubMed: 18832435]
111. Huang J, Niknahad H, Khan S, O'Brien PJ. Hepatocyte-catalysed detoxification of cyanide by Land D-cysteine. Biochem Pharmacol. 1998; 55:1983–90. [PubMed: 9714318]
112. Hultberg B, Agardh E, Andersson A, Brattstrom L, Isaksson A, et al. Increased levels of plasma homocysteine are associated with nephropathy, but not severe retinopathy in type 1 diabetes mellitus. Scand J Clin Lab Invest. 1991; 51:277–82. [PubMed: 1882179]
113. Huxtable RJ. Taurine in the central nervous system and the mammalian actions of taurine. Prog Neurobiol. 1989; 32:471–533. [PubMed: 2664881]
114. Huxtable RJ. Physiological actions of taurine. Physiol Rev. 1992; 72:101–63. [PubMed: 1731369]
115. Iantomasi T, Favilli F, Marraccini P, Magaldi T, Bruni P, Vincenzini MT. Glutathione transport system in human small intestine epithelial cells. Biochim Biophys Acta. 1997; 1330:274–83. [PubMed: 9408181]
116. Irvine JC, Favaloro JL, Widdop RE, Kemp-Harper BK. Nitroxyl anion donor, Angeli's salt, does not develop tolerance in rat isolated aortae. Hypertension. 2007; 49:885–92. [PubMed: 17309955]
117. Irwin MI, Hegsted DM. A conspectus of research on amino acid requirements of man. J Nutr. 1971; 101:539–66. [PubMed: 4931584]
118. Ishii I, Akahoshi N, Yamada H, Nakano S, Izumi T, Suematsu M. Cystathionine γ-lyase-deficient mice require dietary cysteine to protect against acute lethal myopathy and oxidative injury. J Biol Chem. 2010; 285:26358–68. [PubMed: 20566639]
119. Ishii I, Akahoshi N, Yu XN, Kobayashi Y, Namekata K, et al. Murine cystathionine γ-lyase: complete cDNA and genomic sequences, promoter activity, tissue distribution and developmental expression. Biochem J. 2004; 381:113–23. [PubMed: 15038791]
120. Jackson MR, Melideo SL, Jorns MS. Human sulfide:quinone oxidoreductase catalyzes the first step in hydrogen sulfide metabolism and produces a sulfane sulfur metabolite. Biochemistry. 2012; 51:6804–15. [PubMed: 22852582]
121. Jacobs RL, House JD, Brosnan ME, Brosnan JT. Effects of streptozotocin-induced diabetes and of insulin treatment on homocysteine metabolism in the rat. Diabetes. 1998; 47:1967–70. [PubMed: 9836532]
122. Jacobs RL, Stead LM, Brosnan ME, Brosnan JT. Hyperglucagonemia in rats results in decreased plasma homocysteine and increased flux through the transsulfuration pathway in liver. J Biol Chem. 2001; 276:43740–47. [PubMed: 11559709]
123. Jennings ML. Transport of hydrogen sulfide and hydrosulfide anion across the human red blood cell membrane rapid H_2S diffusion and AE1-mediated Cl^{-1}/HS^{-1}exchange. Am J Physiol Cell Physiol. 2013; 305:C941–50. [PubMed: 23864610]
124. Jiang HL, Wu HC, Li ZL, Geng B, Tang CS. Changes of the new gaseous transmitter H_2S in patients with coronary heart disease. Di Yi Jun Yi Da Xue Xue Bao. 2005; 25:951–54. [PubMed: 16109547]
125. Johansen D, Ytrehus K, Baxter GF. Exogenous hydrogen sulfide (H_2S) protects against regional myocardial ischemia-reperfusion injury. Evidence for a role of K_{ATP} channels. Basic Res Cardiol. 2006; 101:53–60. [PubMed: 16328106]
126. Kabil O, Banerjee R. The redox biochemistry of hydrogen sulfide. J Biol Chem. 2010; 285:21903–7. [PubMed: 20448039]
127. Kabil O, Banerjee R. Characterization of patient mutations in human persulfide dioxygenase (ETHE1) involved in H_2S catabolism. J Biol Chem. 2012; 287:44561–67. [PubMed: 23144459]
128. Kabil O, Vitvitsky V, Xie P, Banerjee R. The quantitative significance of the transsulfuration enzymes for H_2S production in murine tissues. Antioxid Redox Signal. 2011; 15:363–72. [PubMed: 21254839]
129. Kabil O, Zhou Y, Banerjee R. Human cystathionine beta-synthase is a target for sumoylation. Biochemistry. 2006; 45:13528–36. [PubMed: 17087506]

130. Kamoun P. Mental retardation in Down syndrome: a hydrogen sulfide hypothesis. Med Hypotheses. 2001; 57:389–92. [PubMed: 11516234]

131. Kamoun P, Belardinelli MC, Chabli A, Lallouchi K, Chadefaux-Vekemans B. Endogenous hydrogen sulfide overproduction in Down syndrome. Am J Med Genet A. 2003; 116A:310–11. [PubMed: 12503113]

132. Kang SS, Wong PW, Susmano A, Sora J, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. Am J Hum Genet. 1991; 48:536–45. [PubMed: 1998339]

133. Kannan R, Mittur A, Bao Y, Tsuruo T, Kaplowitz N. GSH transport in immortalized mouse brain endothelial cells: evidence for apical localization of a sodium-dependent GSH transporter. J Neurochem. 1999; 73:390–99. [PubMed: 10386992]

134. Katori T, Hoover DB, Ardell JL, Helm RH, Belardi DF, et al. Calcitonin gene-related peptide in vivo positive inotropy is attributable to regional sympatho-stimulation and is blunted in congestive heart failure. Circ Res. 2005; 96:234–43. [PubMed: 15591228]

135. Kelleher ZT, Matsumoto A, Stamler JS, Marshall HE. NOS2 regulation of NF-κB by *S*-nitrosylation of p65. J Biol Chem. 2007; 282:30667–72. [PubMed: 17720813]

136. Kilberg MS, Handlogten ME, Christensen HN. Characteristics of system ASC for transport of neutral amino acids in the isolated rat hepatocyte. J Biol Chem. 1981; 256:3304–12. [PubMed: 7204404]

137. Kimura H. Hydrogen sulfide: from brain to gut. Antioxid Redox Signal. 2010; 12:1111–23. [PubMed: 19803743]

138. Kimura H. Hydrogen sulfide: its production, release and functions. Amino Acids. 2011; 41:113–21. [PubMed: 20191298]

139. Kimura Y, Dargusch R, Schubert D, Kimura H. Hydrogen sulfide protects HT22 neuronal cells from oxidative stress. Antioxid Redox Signal. 2006; 8:661–70. [PubMed: 16677109]

140. Kimura Y, Goto Y, Kimura H. Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria. Antioxid Redox Signal. 2010; 12:1–13. [PubMed: 19852698]

141. Kimura Y, Kimura H. Hydrogen sulfide protects neurons from oxidative stress. FASEB J. 2004; 18:1165–67. [PubMed: 15155563]

142. Kirke PN, Molloy AM, Daly LE, Burke H, Weir DG, Scott JM. Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects. Q J Med. 1993; 86:703–8. [PubMed: 8265769]

143. Kletzin A, Urich T, Muller F, Bandeiras TM, Gomes CM. Dissimilatory oxidation and reduction of elemental sulfur in thermophilic archaea. J Bioenerg Biomembr. 2004; 36:77–91. [PubMed: 15168612]

144. Koenitzer JR, Isbell TS, Patel HD, Benavides GA, Dickinson DA, et al. Hydrogen sulfide mediates vasoactivity in an O2-dependent manner. Am J Physiol Heart Circ Physiol. 2007; 292:H1953–60. [PubMed: 17237242]

145. Kombian SB, Reiffenstein RJ, Colmers WF. The actions of hydrogen sulfide on dorsal raphe serotonergic neurons in vitro. J Neurophysiol. 1993; 70:81–96. [PubMed: 8395590]

146. Korendyaseva TK, Kuvatov DN, Volkov VA, Martinov MV, Vitvitsky VM, et al. An allosteric mechanism for switching between parallel tracks in mammalian sulfur metabolism. PLoS Comput Biol. 2008; 4:e1000076. [PubMed: 18451990]

147. Korendyaseva TK, Martinov MV, Dudchenko AM, Vitvitsky VM. Distribution of methionine between cells and incubation medium in suspension of rat hepatocytes. Amino Acids. 2010; 39:1281–89. [PubMed: 20309593]

148. Kraus JP, Janosik M, Kozich V, Mandell R, Shih V, et al. Cystathionine beta-synthase mutations in homocystinuria. Hum Mutat. 1999; 13:362–75. [PubMed: 10338090]

149. Krishnan N, Fu C, Pappin DJ, Tonks NK. H_2S-induced sulfhydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response. Sci Signal. 2011; 4:ra86. [PubMed: 22169477]

150. Kulkarni KH, Monjok EM, Zeyssig R, Kouamou G, Bongmba ON, et al. Effect of hydrogen sulfide on sympathetic neurotransmission and catecholamine levels in isolated porcine iris-ciliary body. Neurochem Res. 2009; 34:400–6. [PubMed: 18629636]

151. Kurpad AV, Regan MM, Varalakshmi S, Gnanou J, Lingappa A, Young VR. Effect of cystine on the methionine requirement of healthy Indian men determined by using the 24-h indicator amino acid balance approach. Am J Clin Nutr. 2004; 80:1526–35. [PubMed: 15585764]
152. Kurpad AV, Regan MM, Varalakshmi S, Vasudevan J, Gnanou J, et al. Daily methionine requirements of healthy Indian men, measured by a 24-h indicator amino acid oxidation and balance technique. Am J Clin Nutr. 2003; 77:1198–205. [PubMed: 12716672]
153. Lash LH, Jones DP. Transport of glutathione by renal basal-lateral membrane vesicles. Biochem Biophys Res Commun. 1983; 112:55–60. [PubMed: 6838619]
154. Lash LH, Jones DP. Renal glutathione transport. Characteristics of the sodium-dependent system in the basal-lateral membrane. J Biol Chem. 1984; 259:14508–14. [PubMed: 6501304]
155. Lash LH, Putt DA, Xu F, Matherly LH. Role of rat organic anion transporter 3 (Oat3) in the renal basolateral transport of glutathione. Chem Biol Interact. 2007; 170:124–34. [PubMed: 17719021]
156. Leclerc D, Wilson A, Dumas R, Gafuik C, Song D, et al. Cloning and mapping of a cDNA for methionine synthase reductase, a flavoprotein defective in patients with homocystinuria. Proc Natl Acad Sci USA. 1998; 95:3059–64. [PubMed: 9501215]
157. Lee JI, Dominy JE Jr, Sikalidis AK, Hirschberger LL, Wang W, Stipanuk MH. HepG2/C3A cells respond to cysteine deprivation by induction of the amino acid deprivation/integrated stress response pathway. Physiol Genomics. 2008; 33:218–29. [PubMed: 18285520]
158. Lee SW, Hu YS, Hu LF, Lu Q, Dawe GS, et al. Hydrogen sulphide regulates calcium homeostasis in microglial cells. Glia. 2006; 54:116–24. [PubMed: 16718684]
159. Leschelle X, Goubern M, Andriamihaja M, Blottiere HM, Couplan E, et al. Adaptative metabolic response of human colonic epithelial cells to the adverse effects of the luminal compound sulfide. Biochim Biophys Acta. 2005; 1725:201–12. [PubMed: 15996823]
160. Levitt MD, Abdel-Rehim MS, Furne J. Free and acid-labile hydrogen sulfide concentrations in mouse tissues: anomalously high free hydrogen sulfide in aortic tissue. Antioxid Redox Signal. 2011; 15:373–78. [PubMed: 20812866]
161. Li L, Bhatia M, Zhu YZ, Zhu YC, Ramnath RD, et al. Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse. FASEB J. 2005; 19:1196–98. [PubMed: 15863703]
162. Li L, Hsu A, Moore PK. Actions and interactions of nitric oxide, carbon monoxide and hydrogen sulphide in the cardiovascular system and in inflammation—a tale of three gases! Pharmacol Ther. 2009; 123:386–400. [PubMed: 19486912]
163. Lim JJ, Liu YH, Khin ES, Bian JS. Vasoconstrictive effect of hydrogen sulfide involves downregulation of cAMP in vascular smooth muscle cells. Am J Physiol Cell Physiol. 2008; 295:C1261–70. [PubMed: 18787076]
164. Lu SC. Glutathione synthesis. Biochim Biophys Acta. 2013; 1830:3143–53. [PubMed: 22995213]
165. Martensson J. The effect of fasting on leukocyte and plasma glutathione and sulfur amino acid concentrations. Metabolism. 1986; 35:118–21. [PubMed: 3945186]
166. Martinov MV, Vitvitsky VM, Banerjee R, Ataullakhanov FI. The logic of the hepatic methionine metabolic cycle. Biochim Biophys Acta. 2010; 1804:89–96. [PubMed: 19833238]
167. Massey V, Edmondson D. On the mechanism of inactivation of xanthine oxidase by cyanide. J Biol Chem. 1970; 245:6595–98. [PubMed: 5536559]
168. Massion PB, Pelat M, Belge C, Balligand JL. Regulation of the mammalian heart function by nitric oxide. Comp Biochem Physiol A Mol Integr Physiol. 2005; 142:144–50. [PubMed: 15985381]
169. Meister A, Anderson ME. Glutathione. Annu Rev Biochem. 1983; 52:711–60. [PubMed: 6137189]
170. Meister A, Fraser PE, Tice SV. Enzymatic desulfuration of beta-mercaptopyruvate to pyruvate. J Biol Chem. 1954; 206:561–75. [PubMed: 13143015]
171. Mikami Y, Shibuya N, Kimura Y, Nagahara N, Ogasawara Y, Kimura H. Thioredoxin and di-hydrolipoic acid are required for 3-mercaptopyruvate sulfurtransferase to produce hydrogen sulfide. Biochem J. 2011; 439:479–85. [PubMed: 21732914]
172. Miller DL, Roth MB. Hydrogen sulfide increases thermotolerance and lifespan in *Caenorhabditis elegans*. Proc Natl Acad Sci USA. 2007; 104:20618–22. [PubMed: 18077331]

173. Miller RA, Buehner G, Chang Y, Harper JM, Sigler R, Smith-Wheelock M. Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. Aging Cell. 2005; 4:119–25. [PubMed: 15924568]
174. Mills JL, McPartlin JM, Kirke PN, Lee YJ, Conley MR, et al. Homocysteine metabolism in pregnancies complicated by neural-tube defects. Lancet. 1995; 345:149–51. [PubMed: 7741859]
175. Mineri R, Rimoldi M, Burlina AB, Koskull S, Perletti C, et al. Identification of new mutations in the *ETHE1* gene in a cohort of 14 patients presenting with ethylmalonic encephalopathy. J Med Genet. 2008; 45:473–78. [PubMed: 18593870]
176. Mirandola P, Gobbi G, Malinverno C, Carubbi C, Ferne FM, et al. Impact of sulphurous water politzer inhalation on audiometric parameters in children with otitis media with effusion. Clin Exp Otorhinolaryngol. 2013; 6:7–11. [PubMed: 23524467]
177. Mitsuhashi H, Ikeuchi H, Yamashita S, Kuroiwa T, Kaneko Y, et al. Increased levels of serum sulfite in patients with acute pneumonia. Shock. 2004; 21:99–102. [PubMed: 14752280]
178. Mitsuhashi H, Yamashita S, Ikeuchi H, Kuroiwa T, Kaneko Y, et al. Oxidative stress-dependent conversion of hydrogen sulfide to sulfite by activated neutrophils. Shock. 2005; 24:529–34. [PubMed: 16317383]
179. Miyazaki T, Matsuzaki Y, Ikegami T, Miyakawa S, Doy M, et al. The harmful effect of exercise on reducing taurine concentration in the tissues of rats treated with CCl4 administration. J Gastroenterol. 2004; 39:557–62. [PubMed: 15235873]
180. Mok YY, Atan MS, Yoke Ping C, Zhong Jing W, Bhatia M, et al. Role of hydrogen sulphide in haemorrhagic shock in the rat: protective effect of inhibitors of hydrogen sulphide biosynthesis. Br J Pharmacol. 2004; 143:881–89. [PubMed: 15504752]
181. Morales I, Dopico JG, Sabate M, Gonzalez-Hernandez T, Rodriguez M. Substantia nigra osmoregulation: taurine and ATP involvement. Am J Physiol Cell Physiol. 2007; 292:C1934–41. [PubMed: 17215320]
182. Moriarty-Craige SE, Jones DP. Extracellular thiols and thiol/disulfide redox in metabolism. Annu Rev Nutr. 2004; 24:481–509. [PubMed: 15189129]
183. Mosharov E, Cranford MR, Banerjee R. The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transsulfuration pathway and its regulation by redox changes. Biochemistry. 2000; 39:13005–11. [PubMed: 11041866]
184. Motl, N.; Yadav, PK.; Banerjee, R. Enzymology of hydrogen sulfide turnover. In: Kimura, H., editor. Hydrogen Sulfide and Its Therapeutic Applications. Wien: Springer-Verlag; 2013. p. 1-35.
185. Mudd SH, Ebert MH, Scriver CR. Labile methyl group balances in the human: the role of sarcosine. Metabolism. 1980; 29:707–20. [PubMed: 6157075]
186. Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, et al. H_2S signals through protein S-sulfhydration. Sci Signal. 2009; 2:ra72. [PubMed: 19903941]
187. Mustafa AK, Sikka G, Gazi SK, Steppan J, Jung SM, et al. Hydrogen sulfide as endothelium-derived hyperpolarizing factor sulfhydrates potassium channels. Circ Res. 2011; 109:1259–68. [PubMed: 21980127]
188. Muyzer G, Stams AJ. The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol. 2008; 6:441–54. [PubMed: 18461075]
189. Myoishi M, Hao H, Minamino T, Watanabe K, Nishihira K, et al. Increased endoplasmic reticulum stress in atherosclerotic plaques associated with acute coronary syndrome. Circulation. 2007; 116:1226–33. [PubMed: 17709641]
190. Nagai Y, Tsugane M, Oka J, Kimura H. Hydrogen sulfide induces calcium waves in astrocytes. FASEB J. 2004; 18:557–59. [PubMed: 14734631]
191. Nahas R, Sheikh O. Complementary and alternative medicine for the treatment of major depressive disorder. Can Fam Phys. 2011; 57:659–63.
192. Nicoletti FP, Thompson MK, Franzen S, Smulevich G. Degradation of sulfide by dehaloperoxidase-hemoglobin from *Amphitrite ornata*. J Biol Inorg Chem. 2011; 16:611–19. [PubMed: 21298302]
193. Nishida M, Sawa T, Kitajima N, Ono K, Inoue H, et al. Hydrogen sulfide anion regulates redox signaling via electrophile sulfhydration. Nat Chem Biol. 2012; 8:714–24. [PubMed: 22772154]

194. Oh GS, Pae HO, Lee BS, Kim BN, Kim JM, et al. Hydrogen sulfide inhibits nitric oxide production and nuclear factor-κB via heme oxygenase-1 expression in RAW264.7 macrophages stimulated with lipopolysaccharide. Free Radic Biol Med. 2006; 41:106–19. [PubMed: 16781459]

195. Olina M, Aluffi Valletti P, Pia F, Toso A, Borello G, et al. Hydrological indications in the therapy of pharyngitis. Recenti Prog Med. 2008; 99:314–21. [PubMed: 18710064]

196. Olson KR. Is hydrogen sulfide a circulating "gasotransmitter" in vertebrate blood? Biochim Biophys Acta. 2009; 1787:856–63. [PubMed: 19361483]

197. Olson KR. A practical look at the chemistry and biology of hydrogen sulfide. Antioxid Redox Signal. 2012; 17:32–44. [PubMed: 22074253]

198. Olson KR. Hydrogen sulfide: both feet on the gas and none on the brake? Front Physiol. 2013; 4:2. [PubMed: 23355823]

199. Olson KR, Dombkowski RA, Russell MJ, Doellman MM, Head SK, et al. Hydrogen sulfide as an oxygen sensor/transducer in vertebrate hypoxic vasoconstriction and hypoxic vasodilation. J Exp Biol. 2006; 209:4011–23. [PubMed: 17023595]

200. Olteanu H, Munson T, Banerjee R. Differences in the efficiency of reductive activation of methionine synthase and exogenous electron acceptors between the common polymorphic variants of human methionine synthase reductase. Biochemistry. 2002; 41:13378–85. [PubMed: 12416982]

201. Orentreich N, Matias JR, DeFelice A, Zimmerman JA. Low methionine ingestion by rats extends life span. J Nutr. 1993; 123:269–74. [PubMed: 8429371]

202. Overmann J, van Gemerden H. Microbial interactions involving sulfur bacteria: implications for the ecology and evolution of bacterial communities. FEMS Microbiol Rev. 2000; 24:591–99. [PubMed: 11077152]

203. Palacin M, Estevez R, Bertran J, Zorzano A. Molecular biology of mammalian plasma membrane amino acid transporters. Physiol Rev. 1998; 78:969–1054. [PubMed: 9790568]

204. Pan J, Carroll KS. Persulfide reactivity in the detection of protein S-sulfhydration. ACS Chem Biol. 2013; 8:1110–16. [PubMed: 23557648]

205. Panza F, Frisardi V, Capurso C, D'Introno A, Colacicco AM, et al. Polyunsaturated fatty acid and *S*-adenosylmethionine supplementation in predementia syndromes and Alzheimer's disease: a review. Sci World J. 2009; 9:373–89.

206. Paolocci N, Saavedra WF, Miranda KM, Martignani C, Isoda T, et al. Nitroxyl anion exerts redox-sensitive positive cardiac inotropy in vivo by calcitonin gene-related peptide signaling. Proc Natl Acad Sci USA. 2001; 98:10463–68. [PubMed: 11517312]

207. Papapetropoulos A, Pyriochou A, Altaany Z, Yang G, Marazioti A, et al. Hydrogen sulfide is an endogenous stimulator of angiogenesis. Proc Natl Acad Sci USA. 2009; 106:21972–77. [PubMed: 19955410]

208. Pascale RM, Simile MM, Gaspa L, Daino L, Seddaiu MA, et al. Alterations of ornithine decarboxylase gene during the progression of rat liver carcinogenesis. Carcinogenesis. 1993; 14:1077–80. [PubMed: 7684954]

209. Patel P, Vatish M, Heptinstall J, Wang R, Carson RJ. The endogenous production of hydrogen sulphide in intrauterine tissues. Reprod Biol Endocrinol. 2009; 7:10. [PubMed: 19200371]

210. Paul BD, Snyder SH. H_2S signalling through protein sulfhydration and beyond. Nat Rev Mol Cell Biol. 2012; 13:499–507. [PubMed: 22781905]

211. Pedersen OO, Karlsen RL. The toxic effect of L-cysteine on the rat retina. A morphological and biochemical study. Invest Ophthalmol Vis Sci. 1980; 19:886–92. [PubMed: 7409983]

212. Perry TL, Hansen S, MacDougall L. Homolanthionine excretion in homocystinuria. Science. 1966; 152:1750–2. [PubMed: 5938411]

213. Petersen LC. The effect of inhibitors on the oxygen kinetics of cytochrome c oxidase. Biochim Biophys Acta. 1977; 460:299–307. [PubMed: 192290]

214. Pfeffer M, Ressler C. Beta-cyanoalanine, an inhibitor of rat liver cystathionase. Biochem Pharmacol. 1967; 16:2299–308. [PubMed: 6075392]

215. Pietri R, Lewis A, Leon RG, Casabona G, Kiger L, et al. Factors controlling the reactivity of hydrogen sulfide with hemeproteins. Biochemistry. 2009; 48:4881–94. [PubMed: 19368335]

216. Pogribna M, Melnyk S, Pogribny I, Chango A, Yi P, James SJ. Homocysteine metabolism in children with Down syndrome: in vitro modulation. Am J Hum Genet. 2001; 69:88–95. [PubMed: 11391481]
217. Powell MA, Somero GN. Hydrogen sulfide oxidation is coupled to oxidative phosphorylation in mitochondria of *Solemya reidi*. Science. 1986; 233:563–66. [PubMed: 17820467]
218. Predmore BL, Kondo K, Bhushan S, Zlatopolsky MA, King AL, et al. The polysulfide diallyl trisulfide protects the ischemic myocardium by preservation of endogenous hydrogen sulfide and increasing nitric oxide bioavailability. Am J Physiol Heart Circ Physiol. 2012; 302:H2410–18. [PubMed: 22467307]
219. Prudova A, Albin M, Bauman Z, Lin A, Vitvitsky V, Banerjee R. Testosterone regulation of homocysteine metabolism modulates redox status in human prostate cancer cells. Antioxid Redox Signal. 2007; 9:1875–81. [PubMed: 17854288]
220. Purohit V, Abdelmalek MF, Barve S, Benevenga NJ, Halsted CH, et al. Role of *S*-adenosylmethionine, folate, and betaine in the treatment of alcoholic liver disease: summary of a symposium. Am J Clin Nutr. 2007; 86:14–24. [PubMed: 17616758]
221. Qabazard B, Li L, Gruber J, Peh MT, Ng LF, et al. Hydrogen sulfide is an endogenous regulator of ageing in *Caenorhabditis elegans*. Antioxid Redox Signal. 2014 In press.
222. Rao AM, Drake MR, Stipanuk MH. Role of the transsulfuration pathway and of gamma-cystathionase activity in the formation of cysteine and sulfate from methionine in rat hepatocytes. J Nutr. 1990; 120:837–45. [PubMed: 2116506]
223. Ratnam S, Maclean KN, Jacobs RL, Brosnan ME, Kraus JP, Brosnan JT. Hormonal regulation of cystathionine β-synthase expression in liver. J Biol Chem. 2002; 277:42912–18. [PubMed: 12198128]
224. Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. Annu Rev Med. 1998; 49:31–62. [PubMed: 9509248]
225. Rej R. Aminooxyacetate is not an adequate differential inhibitor of aspartate aminotransferase isoenzymes. Clin Chem. 1977; 23:1508–9. [PubMed: 872414]
226. Renga B. Hydrogen sulfide generation in mammals: the molecular biology of cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE). Inflamm Allergy Drug Targets. 2011; 10:85–91. [PubMed: 21275900]
227. Richie JP Jr, Komninou D, Leutzinger Y, Kleinman W, Orentreich N, et al. Tissue glutathione and cysteine levels in methionine-restricted rats. Nutrition. 2004; 20:800–5. [PubMed: 15325691]
228. Richman PG, Meister A. Regulation of gamma-glutamyl-cysteine synthetase by nonallosteric feedback inhibition by glutathione. J Biol Chem. 1975; 250:1422–26. [PubMed: 1112810]
229. Riedijk MA, Stoll B, Chacko S, Schierbeek H, Sunehag AL, et al. Methionine transmethylation and transsulfuration in the piglet gastrointestinal tract. Proc Natl Acad Sci USA. 2007; 104:3408–13. [PubMed: 17360659]
230. Robillon JF, Canivet B, Candito M, Sadoul JL, Jullien D, et al. Type 1 diabetes mellitus and homocyst(e)ine. Diabetes Metab. 1994; 20:494–96.
231. Roman HB, Hirschberger LL, Krijt J, Valli A, Kozich V, Stipanuk MH. The cysteine dioxygenase knockout mouse: Altered cysteine metabolism in nonhepatic tissues leads to excess H_2S/HS^{-1}production and evidence of pancreatic and lung toxicity. Antioxid Redox Signal. 2013; 19:1321–36. [PubMed: 23350603]
232. Rose WC, Wixom RL. The amino acid requirements of man. XIII The sparing effect of cystine on the methionine requirement. J Biol Chem. 1955; 216:753–73. [PubMed: 13271351]
233. Roth SH, Skrajny B, Reiffenstein RJ. Alteration of the morphology and neurochemistry of the developing mammalian nervous system by hydrogen sulphide. Clin Exp Pharmacol Physiol. 1995; 22:379–80. [PubMed: 7554437]
234. Rowling MJ, McMullen MH, Chipman DC, Schalinske KL. Hepatic glycine N-methyltransferase is up-regulated by excess dietary methionine in rats. J Nutr. 2002; 132:2545–50. [PubMed: 12221207]

235. Roy A, Khan AH, Islam MT, Prieto MC, Majid DS. Interdependency of cystathione gamma-lyase and cystathione beta-synthase in hydrogen sulfide-induced blood pressure regulation in rats. Am J Hypertens. 2012; 25:74–81. [PubMed: 21866187]
236. Rucklidge JJ, Johnstone J, Kaplan BJ. Nutrient supplementation approaches in the treatment of ADHD. Expert Rev Neurother. 2009; 9:461–76. [PubMed: 19344299]
237. Rudolph TK, Freeman BA. Transduction of redox signaling by electrophile-protein reactions. Sci Signal. 2009; 2:re7. [PubMed: 19797270]
238. Ruiz F, Corrales FJ, Miqueo C, Mato JM. Nitric oxide inactivates rat hepatic methionine adenosyltransferase in vivo by *S*-nitrosylation. Hepatology. 1998; 28:1051–57. [PubMed: 9755242]
239. Salami A, Dellepiane M, Strinati F, Guastini L, Mora R. Sulphurous thermal water inhalations in the treatment of chronic rhinosinusitis. Rhinology. 2010; 48:71–76. [PubMed: 20502739]
240. Sano M, Minamino T, Toko H, Miyauchi H, Orimo M, et al. p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. Nature. 2007; 446:444–48. [PubMed: 17334357]
241. Sato Y, Ouchi K, Funase Y, Yamauchi K, Aizawa T. Relationship between metformin use, vitamin B12 deficiency, hyperhomocysteinemia and vascular complications in patients with type 2 diabetes. Endocr J. 2013; 60:1275–80. [PubMed: 24018893]
242. Sawa T, Zaki MH, Okamoto T, Akuta T, Tokutomi Y, et al. Protein *S*-guanylation by the biological signal 8-nitroguanosine 3′,5′-cyclic monophosphate. Nat Chem Biol. 2007; 3:727–35. [PubMed: 17906641]
243. Scalabrino G, Poso H, Holtta E, Hannonen P, Kallio A, Janne J. Synthesis and accumulation of polyamines in rat liver during chemical carcinogenesis. Int J Cancer. 1978; 21:239–45. [PubMed: 24005]
244. Schreiber G, Schreiber M. Protein synthesis in single cell suspensions from rat liver. I General properties of the system and permeability of the cells for leucine and methionine. J Biol Chem. 1972; 247:6340–46. [PubMed: 4346811]
245. Schuller-Levis GB, Park E. Taurine: new implications for an old amino acid. FEMS Microbiol Lett. 2003; 226:195–202. [PubMed: 14553911]
246. Seelig GF, Simondsen RP, Meister A. Reversible dissociation of gamma-glutamylcysteine synthetase into two subunits. J Biol Chem. 1984; 259:9345–47. [PubMed: 6146611]
247. Sen N, Paul BD, Gadalla MM, Mustafa AK, Sen T, et al. Hydrogen sulfide-linked sulfhydration of NF-κB mediates its antiapoptotic actions. Mol Cell. 2012; 45:13–24. [PubMed: 22244329]
248. Sessa A, Desiderio MA, Baizini M, Perin A. Diamine oxidase activity in regenerating rat liver and in 4-dimethylaminoazobenzene-induced and Yoshida AH 130 hepatomas. Cancer Res. 1981; 41:1929–34. [PubMed: 6783302]
249. Shibuya N, Koike S, Tanaka M, Ishigami-Yuasa M, Kimura Y, et al. A novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells. Nat Commun. 2013; 4:1366. [PubMed: 23340406]
250. Shibuya N, Mikami Y, Kimura Y, Nagahara N, Kimura H. Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces hydrogen sulfide. J Biochem. 2009; 146:623–26. [PubMed: 19605461]
251. Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, et al. 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. Antioxid Redox Signal. 2009; 11:703–14. [PubMed: 18855522]
252. Sikalidis AK, Lee JI, Stipanuk MH. Gene expression and integrated stress response in HepG2/C3A cells cultured in amino acid deficient medium. Amino Acids. 2011; 41:159–71. [PubMed: 20361218]
253. Silverman RB, Abeles RH. Mechanism of inactivation of gamma-cystathionase by beta,beta,beta-trifluoroalanine. Biochemistry. 1977; 16:5515–20. [PubMed: 921947]
254. Singh S, Padovani D, Leslie RA, Chiku T, Banerjee R. Relative contributions of cystathionine beta-synthase and gamma-cystathionase to H_2S biogenesis via alternative trans-sulfuration reactions. J Biol Chem. 2009; 284:22457–66. [PubMed: 19531479]

255. Singh SP, Wishnok JS, Keshive M, Deen WM, Tannenbaum SR. The chemistry of the *S*-nitrosoglutathione/glutathione system. Proc Natl Acad Sci USA. 1996; 93:14428–33. [PubMed: 8962068]
256. Skrajny B, Hannah RS, Roth SH. Low concentrations of hydrogen sulphide alter monoamine levels in the developing rat central nervous system. Can J Physiol Pharmacol. 1992; 70:1515–18. [PubMed: 1296865]
257. Souba WW, Pacitti AJ. How amino acids get into cells: mechanisms, models, menus, and mediators. J Parenter Enteral Nutr. 1992; 16:569–78.
258. Sparatore A, Perrino E, Tazzari V, Giustarini D, Rossi R, et al. Pharmacological profile of a novel H_2S-releasing aspirin. Free Radic Biol Med. 2009; 46:586–92. [PubMed: 19100325]
259. Stamatakis K, Perez-Sala D. Prostanoids with cyclopentenone structure as tools for the characterization of electrophilic lipid-protein interactomes. Ann N Y Acad Sci. 2006; 1091:548–70. [PubMed: 17341644]
260. Stamler JS. *S*-nitrosothiols and the bioregulatory actions of nitrogen oxides through reactions with thiol groups. Curr Top Microbiol Immunol. 1995; 196:19–36. [PubMed: 7634823]
261. Stead LM, Brosnan ME, Brosnan JT. Characterization of homocysteine metabolism in the rat liver. Biochem J. 2000; 350(Part 3):685–92. [PubMed: 10970780]
262. Steegborn C, Clausen T, Sondermann P, Jacob U, Worbs M, et al. Kinetics and inhibition of recombinant human cystathionine gamma-lyase. Toward the rational control of transsulfuration. J Biol Chem. 1999; 274:12675–84. [PubMed: 10212249]
263. Steen C, Rosenblatt DS, Scheying H, Braeuer HC, Kohlschutter A. Cobalamin E (cblE) disease: a severe neurological disorder with megaloblastic anaemia, homocystinuria and low serum methionine. J Inherit Metab Dis. 1997; 20:705–6. [PubMed: 9323567]
264. Stewart FJ, Cavanaugh CM. Symbiosis of thioautotrophic bacteria with *Riftia pachyptila*. Prog Mol Subcell Biol. 2006; 41:197–225. [PubMed: 16623395]
265. Stipanuk MH, Coloso RM, Garcia RA, Banks MF. Cysteine concentration regulates cysteine metabolism to glutathione, sulfate and taurine in rat hepatocytes. J Nutr. 1992; 122:420–27. [PubMed: 1542000]
266. Stipanuk MH, Londono M, Lee JI, Hu M, Yu AF. Enzymes and metabolites of cysteine metabolism in nonhepatic tissues of rats show little response to changes in dietary protein or sulfur amino acid levels. J Nutr. 2002; 132:3369–78. [PubMed: 12421853]
267. Stipanuk MH, Ueki I. Dealing with methionine/homocysteine sulfur: cysteine metabolism to taurine and inorganic sulfur. J Inherit Metab Dis. 2011; 34:17–32. [PubMed: 20162368]
268. Stipanuk MH, Ueki I, Dominy JE Jr, Simmons CR, Hirschberger LL. Cysteine dioxygenase: a robust system for regulation of cellular cysteine levels. Amino Acids. 2009; 37:55–63. [PubMed: 19011731]
269. Sturman JA. Taurine in development. Physiol Rev. 1993; 73:119–47. [PubMed: 8419963]
270. Sturman JA, Messing JM, Gargano AD, Rerecich M, Imaki H, Rudelli R. Cystine neurotoxicity is increased by taurine deficiency. Neurotoxicology. 1989; 10:15–28. [PubMed: 2570388]
271. Szabo C, Ransy C, Modis K, Andriamihaja M, Murghes B, et al. Regulation of mitochondrial bioenergetic function by hydrogen sulfide. Part I Biochemical and physiological mechanisms. Br J Pharmacol. 2014; 171:2099–122. [PubMed: 23991830]
272. Talipov MR, Timerghazin QK. Protein control of *S*-nitrosothiol reactivity: interplay of antagonistic resonance structures. J Phys Chem B. 2013; 117:1827–37. [PubMed: 23316815]
273. Tanase S, Morino Y. Irreversible inactivation of aspartate aminotransferases during transamination with L-propargylglycine. Biochem Biophys Res Commun. 1976; 68:1301–8. [PubMed: 944577]
274. Taoka S, Ohja S, Shan X, Kruger WD, Banerjee R. Evidence for heme-mediated redox regulation of human cystathionine beta-synthase activity. J Biol Chem. 1998; 273:25179–84. [PubMed: 9737978]
275. Thomas T, Thomas TJ. Polyamine metabolism and cancer. J Cell Mol Med. 2003; 7:113–26. [PubMed: 12927050]
276. Timerghazin QK, Peslherbe GH, English AM. Resonance description of *S*-nitrosothiols: insights into reactivity. Org Lett. 2007; 9:3049–52. [PubMed: 17616141]

277. Timerghazin QK, Peslherbe GH, English AM. Structure and stability of HSNO, the simplest *S*-nitrosothiol. Phys Chem Chem Phys. 2008; 10:1532–39. [PubMed: 18327309]

278. Tiranti V, D'Adamo P, Briem E, Ferrari G, Mineri R, et al. Ethylmalonic encephalopathy is caused by mutations in *ETHE1*, a gene encoding a mitochondrial matrix protein. Am J Hum Genet. 2004; 74:239–52. [PubMed: 14732903]

279. Tiranti V, Viscomi C, Hildebrandt T, Di Meo I, Mineri R, et al. Loss of ETHE1, a mitochondrial dioxygenase, causes fatal sulfide toxicity in ethylmalonic encephalopathy. Nat Med. 2009; 15:200–5. [PubMed: 19136963]

280. Tomaskova Z, Bertova A, Ondrias K. On the involvement of H_2S in nitroso signaling and other mechanisms of H_2S action. Curr Pharm Biotechnol. 2011; 12:1394–405. [PubMed: 21235453]

281. Tousoulis D, Papageorgiou N, Androulakis E, Siasos G, Latsios G, et al. Diabetes mellitus-associated vascular impairment: novel circulating biomarkers and therapeutic approaches. J Am Coll Cardiol. 2013; 62:667–76. [PubMed: 23948511]

282. Triguero A, Barber T, Garcia C, Puertes IR, Sastre J, Vina JR. Liver intracellular L-cysteine concentration is maintained after inhibition of the trans-sulfuration pathway by propargylglycine in rats. Br J Nutr. 1997; 78:823–31. [PubMed: 9389904]

283. Troen AM, Lutgens E, Smith DE, Rosenberg IH, Selhub J. The atherogenic effect of excess methionine intake. Proc Natl Acad Sci USA. 2003; 100:15089–94. [PubMed: 14657334]

284. Tuz K, Ordaz B, Vaca L, Quesada O, Pasantes-Morales H. Isovolumetric regulation mechanisms in cultured cerebellar granule neurons. J Neurochem. 2001; 79:143–51. [PubMed: 11595766]

285. Ubuka T, Ohta J, Yao WB, Abe T, Teraoka T, Kurozumi Y. L-Cysteine metabolism via 3-mercaptopyruvate pathway and sulfate formation in rat liver mitochondria. Amino Acids. 1992; 2:143–55. [PubMed: 24194282]

286. Ubuka T, Umemura S, Yuasa S, Kinuta M, Watanabe K. Purification and characterization of mitochondrial cysteine aminotransferase from rat liver. Physiol Chem Phys. 1978; 10:483–500. [PubMed: 754189]

287. Uchida K, Shibata T. 15-Deoxy-Delta(12,14)-prostaglandin J2: an electrophilic trigger of cellular responses. Chem Res Toxicol. 2008; 21:138–44. [PubMed: 18052108]

288. Ueki I, Roman HB, Valli A, Fieselmann K, Lam J, et al. Knockout of the murine cysteine dioxygenase gene results in severe impairment in ability to synthesize taurine and an increased catabolism of cysteine to hydrogen sulfide. Am J Physiol Endocrinol Metab. 2011; 301:E668–84. [PubMed: 21693692]

289. Viscomi C, Burlina AB, Dweikat I, Savoiardo M, Lamperti C, et al. Combined treatment with oral metronidazole and *N*-acetylcysteine is effective in ethylmalonic encephalopathy. Nat Med. 2010; 16:869–71. [PubMed: 20657580]

290. Vitvitsky V, Dayal S, Stabler S, Zhou Y, Wang H, et al. Perturbations in homocysteine-linked redox homeostasis in a murine model for hyperhomocysteinemia. Am J Physiol Regul Integr Comp Physiol. 2004; 287:R39–46. [PubMed: 15016621]

291. Vitvitsky V, Garg SK, Banerjee R. Taurine biosynthesis by neurons and astrocytes. J Biol Chem. 2011; 286:32002–10. [PubMed: 21778230]

292. Vitvitsky V, Kabil O, Banerjee R. High turnover rates for hydrogen sulfide allow for rapid regulation of its tissue concentrations. Antioxid Redox Signal. 2012; 17:22–31. [PubMed: 22229551]

293. Vitvitsky V, Martinov M, Ataullakhanov F, Miller RA, Banerjee R. Sulfur-based redox alterations in long-lived Snell dwarf mice. Mech Ageing Dev. 2013; 134:321–30. [PubMed: 23707637]

294. Vitvitsky V, Mosharov E, Tritt M, Ataullakhanov F, Banerjee R. Redox regulation of homocysteine-dependent glutathione synthesis. Redox Rep. 2003; 8:57–63. [PubMed: 12631446]

295. Vitvitsky V, Prudova A, Stabler S, Dayal S, Lentz SR, Banerjee R. Testosterone regulation of renal cystathionine beta-synthase: implications for sex-dependent differences in plasma homocysteine levels. Am J Physiol Ren Physiol. 2007; 293:F594–600.

296. Vitvitsky V, Thomas M, Ghorpade A, Gendelman HE, Banerjee R. A functional transsulfuration pathway in the brain links to glutathione homeostasis. J Biol Chem. 2006; 281:35785–93. [PubMed: 17005561]

297. Vorobets VS, Kovach SK, Kolbasov GY. Distribution of ion species and formation of ion pairs in concentrated polysulfide solutions in photoelectrochemical transducers. Russ J Appl Chem. 2002; 75:229–34.

298. Wagner CA, Lang F, Broer S. Function and structure of heterodimeric amino acid transporters. Am J Physiol Cell Physiol. 2001; 281:C1077–93. [PubMed: 11546643]

299. Wang R. Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. Physiol Rev. 2012; 92:791–896. [PubMed: 22535897]

300. Wang T, Wang Q, Wang Z, Xiao Z, Liu L. Diagnostic value of the combined measurement of serum Hcy, serum Cys C, and urinary microalbumin in type 2 diabetes mellitus with early complicating diabetic nephropathy. ISRN Endocrinol. 2013; 2013:407452. [PubMed: 24159393]

301. Wang Z, Liu DX, Wang FW, Zhang Q, Du ZX, et al. L-Cysteine promotes the proliferation and differentiation of neural stem cells via the CBS/H(2)S pathway. Neuroscience. 2013; 237:106–17. [PubMed: 23376738]

302. Warenycia MW, Smith KA, Blashko CS, Kombian SB, Reiffenstein RJ. Monoamine oxidase inhibition as a sequel of hydrogen sulfide intoxication: increases in brain catecholamine and 5-hydroxytryptamine levels. Arch Toxicol. 1989; 63:131–36. [PubMed: 2730337]

303. Warskulat U, Heller-Stilb B, Oermann E, Zilles K, Haas H, et al. Phenotype of the taurine transporter knockout mouse. Methods Enzymol. 2007; 428:439–58. [PubMed: 17875433]

304. Waterland RA. Assessing the effects of high methionine intake on DNA methylation. J Nutr. 2006; 136:1706–10S.

305. Watkins D, Rosenblatt DS. Genetic heterogeneity among patients with methylcobalamin deficiency. Definition of two complementation groups, cblE and cblG. J Clin Invest. 1988; 81:1690–94. [PubMed: 3384945]

306. Watkins D, Rosenblatt DS. Functional methionine synthase deficiency (cblE and cblG): clinical and biochemical heterogeneity. Am J Med Genet. 1989; 34:427–34. [PubMed: 2688421]

307. Whiteman M, Le Trionnaire S, Chopra M, Fox B, Whatmore J. Emerging role of hydrogen sulfide in health and disease: critical appraisal of biomarkers and pharmacological tools. Clin Sci. 2011; 121:459–88. [PubMed: 21843150]

308. Whiteman M, Li L, Kostetski I, Chu SH, Siau JL, et al. Evidence for the formation of a novel nitrosothiol from the gaseous mediators nitric oxide and hydrogen sulphide. Biochem Biophys Res Commun. 2006; 343:303–10. [PubMed: 16540095]

309. Whitfield NL, Kreimier EL, Verdial FC, Skovgaard N, Olson KR. Reappraisal of H_2S/sulfide concentration in vertebrate blood and its potential significance in ischemic preconditioning and vascular signaling. Am J Physiol Regul Integr Comp Physiol. 2008; 294:R1930–37. [PubMed: 18417642]

310. Wilson A, Platt R, Wu Q, Leclerc D, Christensen B, et al. A common variant in methionine synthase reductase combined with low cobalamin (vitamin B12) increases risk for spina bifida. Mol Genet Metab. 1999; 67:317–23. [PubMed: 10444342]

311. Wong PS, Hyun J, Fukuto JM, Shirota FN, DeMaster EG, et al. Reaction between *S*-nitrosothiols and thiols: generation of nitroxyl (HNO) and subsequent chemistry. Biochemistry. 1998; 37:5362–71. [PubMed: 9548918]

312. Wu C, Miloslavskaya I, Demontis S, Maestro R, Galaktionov K. Regulation of cellular response to oncogenic and oxidative stress by Seladin-1. Nature. 2004; 432:640–45. [PubMed: 15577914]

313. Wu L, Yang W, Jia X, Yang G, Duridanova D, et al. Pancreatic islet overproduction of H_2S and suppressed insulin release in Zucker diabetic rats. Lab Invest. 2009; 89:59–67. [PubMed: 19002107]

314. Yadav PK, Xie P, Banerjee R. Allosteric communication between the pyridoxal 5′-phosphate (PLP) and heme sites in the H_2S generator human cystathionine β-synthase. J Biol Chem. 2012; 287:37611–20. [PubMed: 22977242]

315. Yadav PK, Yamada K, Chiku T, Koutmos M, Banerjee R. Structure and kinetic analysis of H_2S production by human mercaptopyruvate sulfurtransferase. J Biol Chem. 2013; 288:20002–13. [PubMed: 23698001]

316. Yan Z, Banerjee R. Redox remodeling as an immunoregulatory strategy. Biochemistry. 2010; 49:1059–66. [PubMed: 20070126]

317. Yang G, Sun X, Wang R. Hydrogen sulfide-induced apoptosis of human aorta smooth muscle cells via the activation of mitogen-activated protein kinases and caspase-3. FASEB J. 2004; 18:1782–84. [PubMed: 15371330]

318. Yang G, Wu L, Bryan S, Khaper N, Mani S, Wang R. Cystathionine gamma-lyase deficiency and overproliferation of smooth muscle cells. Cardiovasc Res. 2010; 86:487–95. [PubMed: 20051385]

319. Yang G, Wu L, Jiang B, Yang W, Qi J, et al. H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. Science. 2008; 322:587–90. [PubMed: 18948540]

320. Yong QC, Choo CH, Tan BH, Low CM, Bian JS. Effect of hydrogen sulfide on intracellular calcium homeostasis in neuronal cells. Neurochem Int. 2010; 56:508–15. [PubMed: 20026367]

321. Yong QC, Hu LF, Wang S, Huang D, Bian JS. Hydrogen sulfide interacts with nitric oxide in the heart: possible involvement of nitroxyl. Cardiovasc Res. 2010; 88:482–91. [PubMed: 20660605]

322. Yong R, Searcy DG. Sulfide oxidation coupled to ATP synthesis in chicken liver mitochondria. Comp Biochem Physiol B Biochem Mol Biol. 2001; 129:129–37. [PubMed: 11337256]

323. Yu YB, Yang SF. Auxin-induced ethylene production and its inhibition by aminoethyoxyvinyl-glycine and cobalt ion. Plant Physiol. 1979; 64:1074–77. [PubMed: 16661095]

324. Yusuf M, Kwong Huat BT, Hsu A, Whiteman M, Bhatia M, Moore PK. Streptozotocin-induced diabetes in the rat is associated with enhanced tissue hydrogen sulfide biosynthesis. Biochem Biophys Res Commun. 2005; 333:1146–52. [PubMed: 15967410]

325. Zaghini G, Biagi G. Nutritional peculiarities and diet palatability in the cat. Vet Res Commun. 2005; 29(Suppl 2):39–44. [PubMed: 16244923]

326. Zhang Y, Tang ZH, Ren Z, Qu SL, Liu MH, et al. Hydrogen sulfide, the next potent preventive and therapeutic agent in aging and age-associated diseases. Mol Cell Biol. 2013; 33:1104–13. [PubMed: 23297346]

327. Zhao W, Wang R. H_2S-induced vasorelaxation and underlying cellular and molecular mechanisms. Am J Physiol Heart Circ Physiol. 2002; 283:H474–80. [PubMed: 12124191]

328. Zhao W, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener. EMBO J. 2001; 20:6008–16. [PubMed: 11689441]

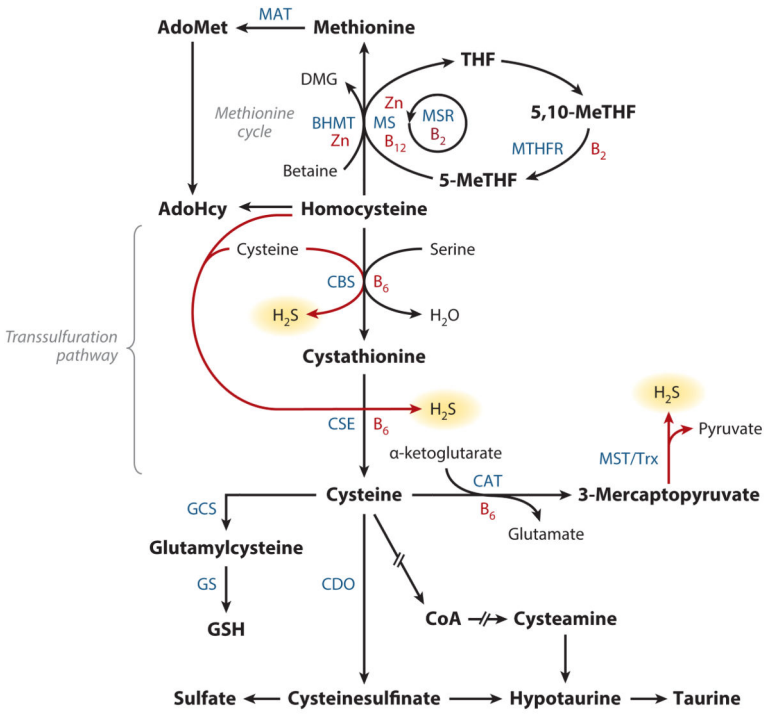

Figure 1.

Simplified scheme of sulfur metabolism with an emphasis on the H_2S-generating reactions. In addition to the amino acids methionine and cysteine, the other significant dietary inputs into the pathway are the vitamins B_2 (flavin), B_6 (pyridoxal phosphate), and B_{12} and zinc. Abbreviations: AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; BHMT, betaine homocysteine methyltransferase; CAT, cysteine/aspartate aminotransferase; CBS, cystathionine β-synthase; CDO, cysteine dioxygenase; CoA, coenzyme A; CSE, γ-cystathionase; DMG, dimethylglycine; GCS, γ-glutamylcysteine synthetase; GS, glutathione synthetase; GSH, glutathione; H_2S, hydrogen sulfide; MAT, methionine adenosyltransferase; MeTHF, 5-methyltetrahydrofolate; MS, methionine synthase; MSR, methionine synthase reductase; MST/Trx, mercaptopyruvate sulfurtransferase/thioredoxin; MTHFR, methylenetetrahydrofolate reductase; THF, tetrahydrofolate; Trx, thioredoxin; Zn, zinc.

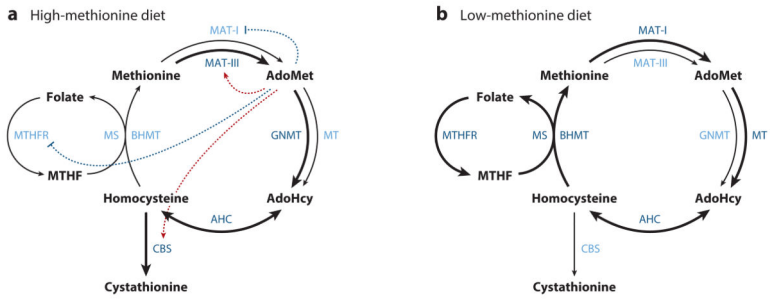

Figure 2.

Switching of methionine metabolism between the disposal (*a*) and conservation (*b*) modes is regulated by AdoMet. The thick arrows qualitatively depict increased metabolic flux. The dashed lines indicate activation (*red*) and inhibition (*blue*) by AdoMet to control flux in response to low and high methionine supply. Abbreviations: AHC, AdoHcy hydrolase; GNMT, glycine *N*-methyltransferase; MAT-I/III, liver-specific methionine adenosyltransferase isoforms I and III; MT, methyltransferase.

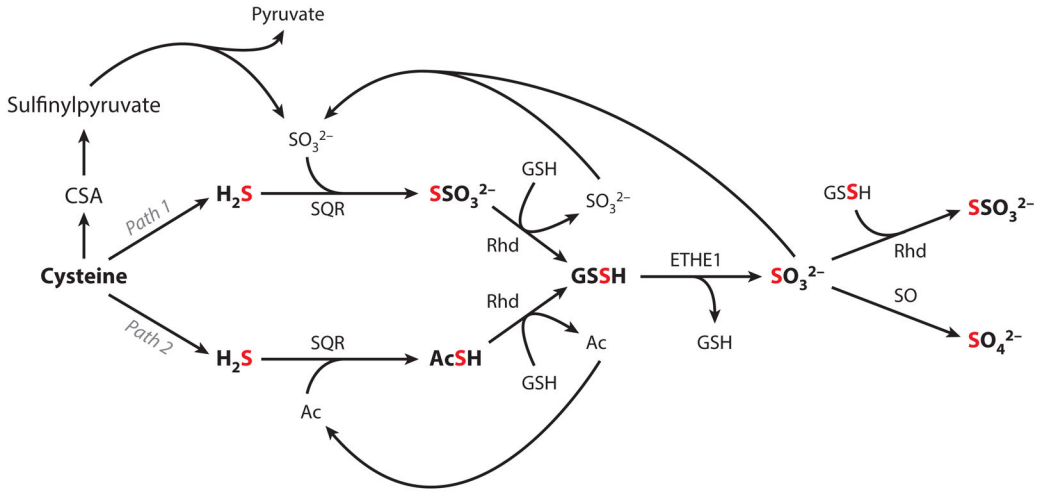

Figure 3.

The mitochondrial sulfide oxidation pathway. Sulfide quinone oxidoreductase (SQR) constitutes the first enzyme in this pathway and oxidizes H_2S, generating a protein-bound persulfide. Two routes for the transfer of the persulfide to sulfite (*path 1*) or to an alternative acceptor (Ac in *path 2*) are shown. In path 1, the thiosulfate formed in the SQR-catalyzed reaction has to be converted to glutathione disulfide (GSSH) for further oxidation by ethylmalonic encephalopathy 1 (ETHE1). In path 2, if glutathione (GSH) is the acceptor, then the resulting GSSH product can directly serve as the substrate for ETHE1. However, if some other molecule serves as the proximal persulfide acceptor from SQR, then a sulfurtransferase (ST) such as rhodanese (Rhd) would likely be necessary to transfer the persulfide group to GSH for further oxidation. CSA and SO denote cysteine sulfinic acid and sulfite oxidase, respectively.

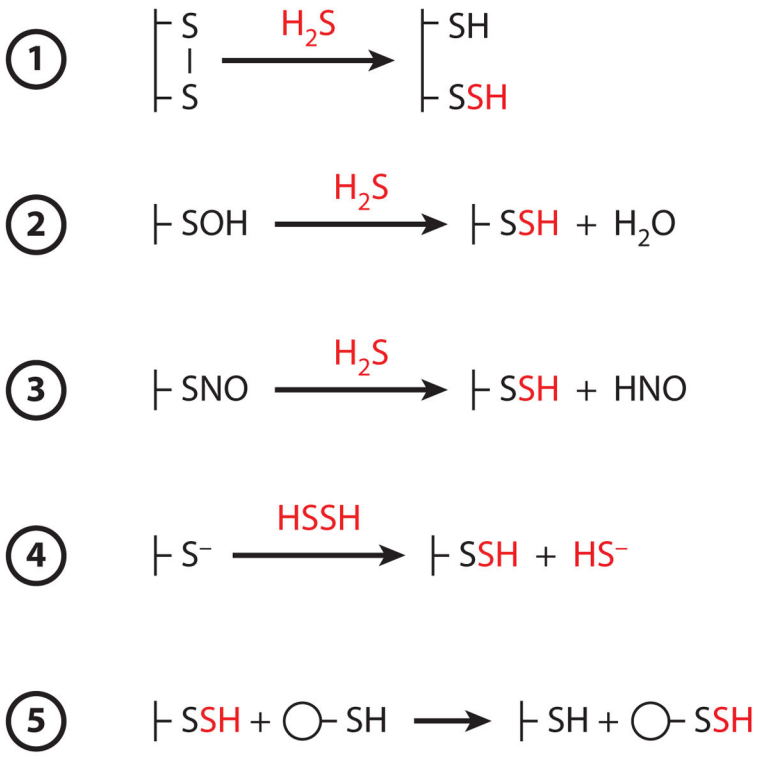

Figure 4.

Molecular mechanisms for protein persulfidation. Persulfidation of targets can occur via several mechanisms, including ① a nucleophilic attack of sulfide on a disulfide, ② cysteine sulfenic acid, or ③ cysteine-*S*-nitrosothiol; ④ a reaction between a protein thiolate and H_2S_2 or polysulfide; or ⑤ a transsulfuration reaction in which the sulfane sulfur from an existing persulfide is transferred to a different acceptor. Abbreviations: HNO, nitroxyl; HSSH, hydrogen disulfide; SNO, *S*-nitrosocysteine; SOH, sulfenic acid; SSH, persulfide.

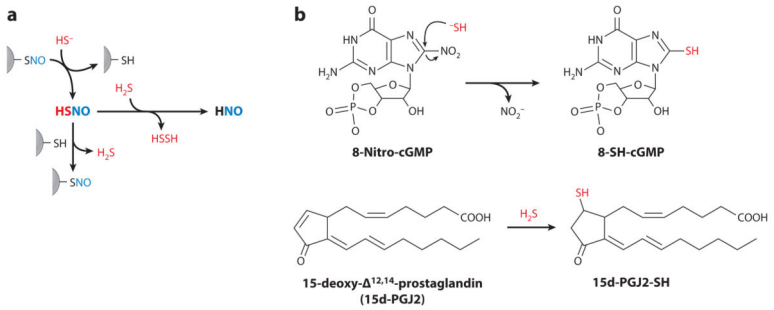

Figure 5.

Potential molecular mechanisms for H_2S signaling via *S*-nitrosothiol (HSNO) or sulfhydration of electrophiles. (*a*) Formation of HSNO results in cross talk between the NO and H_2S signaling pathways. HSNO can mediate transnitrosylation directly or can react further with another mole of H_2S, generating HNO. (*b*) Sulfhydration of electrophiles 8-nitro-cGMP and 15-deoxy-Δ12,14-prostaglandin (15d-PGJ2) by H_2S forms the corresponding thio adducts. Abbreviations: HNO, nitroxyl; HSSH, hydrogen disulfide; SNO, *S*-nitrosocysteine.

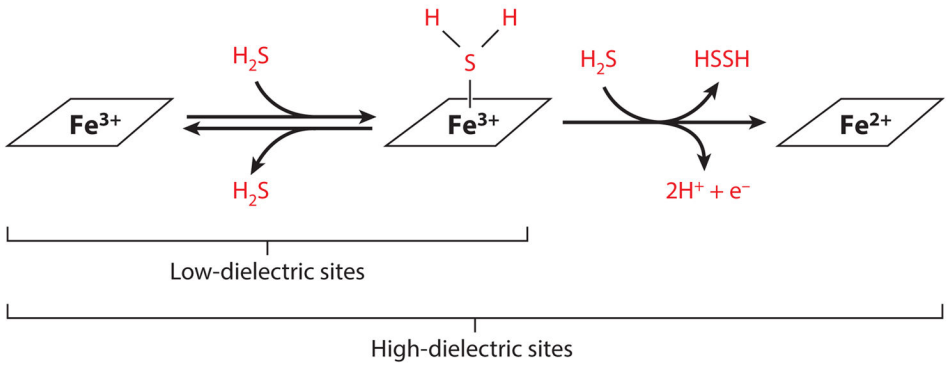

Figure 6.

Reaction of H_2S with heme. The product of the interaction between H_2S and ferric heme is dictated by the properties of the heme pocket. Although H_2S coordinates to the ferric heme ion without additional redox chemistry in polar (high-dielectric-constant) sites, reduction to the ferrous heme is favored in nonpolar (low-dielectric-constant) sites.

Table 1

H_2S-generating reactions catalyzed by CBS and CSE

Enzyme	Substrates	Products
CBS[1]	Cysteine	Serine + H_2S
	Cysteine + homocysteine	Cystathionine + H_2S
	Cysteine + cysteine	Lanthionine + H_2S
CSE[2]	Cysteine	Pyruvate + H_2S + NH_3
	Cysteine + cysteine	Lanthionine + H_2S
	Homocysteine	α-Ketobutyrate + H_2S + NH_3
	Homocysteine + homocysteine	Homolanthionine + H_2S
	Homocysteine + cysteine	Cystathionine + H_2S

[1] Taken from (254);

[2] taken from (43). Abbreviations: CBS, cystathionine β-synthase; CSE, γ-cystathionase; H_2S, hydrogen sulfide; NH_3, ammonia.