Critical Review

Hydrogen Sulfide in Physiology and Pathogenesis of Bacteria and Viruses

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Abstract

An increasing number of studies have established hydrogen sulfide (H2S) gas as a major cytoprotectant and redox modulator. Following its discovery, H2S has been found to have pleiotropic effects on physiology and human health. H2S acts as a gasotransmitter and exerts its influence on gastrointestinal, neuronal, cardiovascular, respiratory, renal, and hepatic systems. Recent discoveries have clearly indicated the importance of H2S in regulating vasorelaxation, angiogenesis, apoptosis, ageing, and metabolism. Contrary to studies in higher organisms, the role of H2S in the pathophysiology of infectious agents such as bacteria and viruses has been less studied. Bacterial and viral infections are often accompanied by changes in the redox physiology of both the host and the pathogen. Emerging studies indicate that bacterial-derived H2S constitutes a defense system against antibiotics and oxidative stress. The H2S signaling pathway also seems to interfere with redox-based events affected on infection with viruses. This review aims to summarize recent advances on the emerging role of H2S gas in the bacterial physiology and viral infections. Such studies have opened up new research avenues exploiting H2S as a potential therapeutic intervention.

Keywords: hydrogen sulfide; cytoprotectant; antioxidant; metabolism; infection; Mycobacterium tuberculosis; HIV.

Abbreviations: CBS, Cystathionine beta synthase; CSE, Cystathionine gamma lyase; 3-MST, 3-mercaptopyruvate sulfurtransferase; SOR, Sulfide quinone oxidoreductase; LTBP, Long term potentiation; NMDA, N-methyl-D-aspartate; GSH, Glutathione; RSV, Respiratory syncytial virus; HIV-1, Human immunodeficiency virus type 1; PLP, Pyridoxal-5-phosphate; SAM, S-adenosyl methionine; DAO, D-amino oxidase; 3-MP, 3-mercaptopyruvate; ox-PMT, Oxidative posttranslational modification; ROS, Reactive oxygen species; RNS, Reactive nitrogen species; ROI, Reactive oxygen intermediates; CcO, Cytochrome c oxidase; SOD, Superoxide dismutase; OCR, Oxygen consumption rate; LC-MS, Liquid chromatography - Mass spectrometry; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; Nrf2, Nuclear factor-erythroid-2 p45 related factor 2; ARE, Antioxidant response element; Keap1, Kelch-like ECH-associated protein 1; NF-kB, Nuclear factor kappa-light-chain-enhancer of activated B cell; GC, Gas chromatography; HPLC, High pressure liquid chromatography; FAM, Fluorescein; GFP, Green fluorescent protein; pAzF, para-Azido phenylalanine; MMTS, S-methyl methanethiosulfonate; IAA, Iodoacetic acid; IAM, Iodoacetamide; DTT, Dithiothreitol; CydB, Cytochrome bd oxidase; CyaA, Cytochrome bo oxidase; MSH, Mycothiol; MSSM, Oxidized mycothiol; RNA, Ribonucleic acid; DNA, Deoxyribonucleic acid; Trx, Thioredoxin; PBMC, Peripheral blood mononuclear cells; AP-1, Activator protein 1; Sp1, Specificity protein 1; LTR, Long terminal repeat; roGFP, Redox-sensitive green fluorescent protein; NAC, N-acetylcysteine; SRB, Sulfur reducing bacteria; UC, Ulcerative colitis; TFF3, Trefoil factor 3; IRF3, Interferon regulatory factor; MAPK, Mitogen-activated protein kinase; ERK, Extracellular signal regulated kinase; JNK, c-Jun N-terminal kinase

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INTRODUCTION

Early life forms first appeared on an anoxic earth in the Archean eon, approximately 3.8 billion years ago (1,2). Among them, were the dissimilatory sulfate-reducing bacteria which constitute one of the oldest forms of bacterial life on earth. These bacteria utilized inorganic sulfur substrates and produced hydrogen sulfide (H₂S) as the end product of anaerobic respiration (3). Before the “great oxidation event” which occurred 2.5 billion years ago leading to an increase in atmospheric oxygen, H₂S remained the most abundant and versatile chemical on the primitive earth (4,5). In fact, H₂S is widely believed to be the primordial sustainable energy source (6). Primitive photolithotrophs used sulfide as the terminal electron acceptor, similar to today’s green and purple sulfur bacteria. Therefore, sulfide-based metabolism may have preceded the present, oxygen-based life on the planet by billions of years (7–9).

In sharp contrast to its pivotal role early in the evolutionary timeline, H₂S is known mostly for being a foul smelling poisonous gas, associated with sewers, septic tanks, and as a weapon of chemical warfare during the First World War. Consequently, majority of the research pertaining to this gas has been conducted from a toxicology point of view (10,11). With studies published as long back as 1803 highlighting the detrimental effect of H₂S on animals, along with the recent gene expression data, H₂S was condemned as a respiratory and metabolic poison (12). It was not until the 1940s that the “transsulfuration” pathway involving the production of H₂S by interconversion between cysteine, homocysteine via cystathionine was described for the first time in liver homogenates (13,14). Further studies led to detailed biochemical characterization of the enzymes cystathionine beta synthase (CBS) and cystathionine gamma lyase (CSE) involved in the transsulfuration reaction. Later, another enzyme, 3-mercaptopyruvate sulfurtansferase (3-MST) was identified as a part of H₂S biogenesis pathways (15–18). However, the functional implication of the H₂S biogenesis remained elusive for a long time. First glimpse of H₂S involvement in cellular physiology emerged from the studies demonstrating measurable levels of endogenous H₂S within brain tissues of healthy individuals (0.65–0.73 μg/g) and animals (1.57 ± 0.04 μg/g) (19–21). Along these lines, higher levels of neuronal H₂S was found to be due to greater expression of CBS in the brain tissues. Additionally, H₂S production in the brain tissue was efficiently reduced using pharmacological CBS inhibitors (hydroxylamine and aminoxyacetic acid). Further studies proposed that H₂S facilitates the induction of hippocampal long-term potentiation (LTP) by enhancing the activity of N-methyl-D-aspartate (NMDA) receptors (22). Later, H₂S was found to relax vascular smooth muscle by activating ATP-sensitive K⁺, intermediate conductance Ca²⁺-sensitive K⁺, and small conductance Ca²⁺-sensitive K⁺ channels (23–25). Importantly, H₂S was identified to protect from oxidative stress and ischemia-reperfusion injury by multiple mechanisms such as restoring the levels of GSH and direct scavenging of mitochondrial ROS (Fig. 1) (26,27). These discoveries further led to the disclosure of mechanisms by which H₂S protects various organs, including the heart and kidney from oxidative stress and ischemia-reperfusion injury (28). Based on these studies, H₂S was inducted as the newest member of the mammalian system.

**Molecular targets of H₂S in mammalian system.** Top left: H₂S has the ability to directly scavenge reactive oxygen and nitrogen species (ROS/RNS). Top right: H₂S targets metal cofactor of cytochrome c oxidase and leads to inhibition of cellular respiration. Oxidation of H₂S by sulfide quinone oxidoreductase (SQO) couples catabolism of H₂S with mitochondrial electron transport chain (ETC) and thus modulates cellular metabolism. Bottom left: ion channels which are involved in systemic responses to H₂S in blood vessels, heart, and neurons. Bottom right: H₂S targets cysteine thiol by S-persulfidation of intracellular signaling proteins and transcription factors which likely accounts for the downstream effects on inflammation, antioxidant response, cellular proliferation, and survival.

![Molecular targets of H₂S in mammalian system](image-url)
of the family of small molecule gaseous transmitters or “gaseotransmitters” alongside nitric oxide (NO) and carbon monoxide (CO) (29). Along this line hydrogen gas (H₂) has also emerged as a potential gaseous signaling molecule with therapeutic antioxidant function (30,31). Recently, H₂S was found to have a protective role in airway epithelial cells infected with respiratory syncytial virus (RSV) demonstrating for the first time that this molecule might be used as a therapeutic agent. Needless to say, the role of H₂S has permeated to areas of metabolism, redox physiology, neurophysiology, apoptosis, angiogenesis, ageing, inflammation, atherosclerosis, pulmonary diseases among others with a whole spectrum of physiological implications (32–34).

H₂S producing bacteria were discovered way back in 1877. Many investigators demonstrated bacterial production of H₂S by its rotten egg smell and its ability to react with lead acetate resulting in the blackening of paper strips impregnated with lead acetate (12,35). In fact, lead acetate test was successfully exploited to distinguish between the paratyphoid and enteritidis groups and still remains an indispensable diagnostic tool (36). In the area of marine microbiology, H₂S emitted from deep sea vents is often referred to as the “sunlight of the deep ocean” (37,38). H₂S forms an important source of metabolic energy for the microorganisms inhabiting such niches, reminiscent of the primordial earth (6). Many bacterial species were demonstrated to possess orthologs of the transsulfuration pathway enzymes namely cystathionine beta synthase or CBS (EC 4.2.1.22) and cystathionine gamma lyase or CSE (EC 4.4.1.1). Both the enzymes uses pyridoxal phosphate (PLP)
A new pathway to generate H$_2$S using D-cysteine has been identified. The enzymes D-amino acid oxidase (DAO) along with 3-MST to form serine and H$_2$S are frequently desulfurated by 3-mercaptopyruvate sulfurtransferase (3-MST) to form H$_2$S (Fig. 3) (45). More recently, a new pathway to generate H$_2$S using D-cysteine has been identified. The enzymes D-amino acid oxidase (DAO) along with 3-MST carry out biogenesis of H$_2$S from D-cysteine. However, when serine is replaced by cysteine, H$_2$S is produced (Fig. 3). CBS catalyses the first and committed step of the transsulfuration pathway which canically, leads to the production of cystathionine from serine and homocysteine (52). However, when serine is replaced by cysteine, H$_2$S is produced (Fig. 3). CBS catalyses the first and committed step of the transsulfuration pathway which canically, leads to the production of cystathionine from serine and homocysteine (52). However, when serine is replaced by cysteine, H$_2$S is produced (Fig. 3).

**Pathways involved in enzymatic biogenesis of H$_2$S.** The transsulfuration pathway consisting of the enzymes cystathionine β-synthase (CBS) and Cystathionine γ-lyase (CSE) is the major pathway for biological H$_2$S production. In addition to this 3-mercaptopyruvate sulfurtransferase/cysteine aminotransferase (3-MST/CAT) pathway also contribute significantly to the production of H$_2$S.

Cystathionine Beta Synthase

Human cystathionine beta synthase (CBS) is a tetramer and is allosterically stimulated by S-adenosyl methionine (SAM) which binds to a conserved “CBS pair domain” in the C-terminal end of the protein (50,51). CBS catalyses the first and committed step of the transsulfuration pathway which canically, leads to the production of cystathionine from serine and homocysteine (52). However, when serine is replaced by cysteine, H$_2$S is produced (Fig. 3). CBS catalyses the first and committed step of the transsulfuration pathway which canically, leads to the production of cystathionine from serine and homocysteine (52). However, when serine is replaced by cysteine, H$_2$S is produced (Fig. 3).

**Cystathionine Gamma Lyase**

As the name suggests, the second enzyme of the pathway CSE, primarily catalyses the cleavage of cystathionine to form cysteine, ammonia and α-ketobutyrate (52). Human CSE is a homotetramer and a PLP binding protein. It can catalyse the production of H$_2$S from cysteine and homocysteine alone or in combination (Fig. 3). The substrate promiscuity of CSE permits the accommodation of cysteine, homocysteine, and cystathionine in the same binding pocket. Regulation of CSE is not very well known but as mentioned earlier, on ER stress it upregulates the production of H$_2$S by utilizing cysteine and homocysteine over cystathionine as substrates (55).

**Mercaptopyruvate Sulfurtransferase.** 3-mercaptoppyruvate is generated by a transamination reaction between cysteine and α-ketoglutarate catalysed by aspartate/cysteine aminotransferase (56). This 3 mercaptoppyruvate (3-MP) is subsequently used as a substrate by 3-MST to form H$_2$S (Fig. 3). 3-MST transfers the sulfur to a nucleophilic cysteine in the active site. This leads to the formation of a bound persulfide which acts as a source of H$_2$S under reducing conditions or in presence of acceptors like thioredoxin. 3-MST is localized to the mitochondria unlike CBS and CSE which are cytosolic, where it is believed to contribute bioenergetically via sulfide oxidation. Also, unlike the transsulfuration enzymes, 3-MST is inhibited under oxidizing conditions due to a labile active site cysteine which gets converted to cysteine sulfonate leading to enzyme inactivation (57).

**MST/DAO Pathway.** In addition to L-cysteine, H$_2$S production was observed in brain homogenates when D-cysteine was used as a substrate. This led to the discovery of new pathway involving peroxisomal enzyme D-amino oxidase (DAO) in H$_2$S biogenesis. D-cysteine is metabolized by DAO to 3-mercaptoppyruvate (3MP), which then translocates to mitochondria where it is converted to H$_2$S and pyruvate. It has been reported that the production of H$_2$S from D-cysteine is ~ 60 times greater in comparison to L-cysteine. Since DAO is only localized to the brain and the kidney, the functionality of the 3MST/DAO pathway for the production of H$_2$S is only relevant to these tissues (49,58).
redox potentials (62–65). While the physiological concentration of major cellular antioxidant buffers, glutathione disulfide/glutathione, versus the standard hydrogen electrode is comparable to that of H2S. Alternatively, it is proposed that H2S can modulate intracellular oxidative stress by acting as an antioxidant buffer (46).

Table 1. Second-order rate constants of H2S with various oxidants have been summarized in Table 1. While these studies indicate a direct scavenging of oxidants by H2S in vitro, the low concentrations of H2S (10 nM to 3 μM) compared to other antioxidants (GSH; 1–10 mM) in vivo raised substantial concerns about its role in remediating ROS/RNS under biologically relevant conditions (66,76–79). Alternatively, H2S has been shown to increase GSH production by enhancing the inward transport of cystine and inducing the expression of GSH-biosynthetic enzyme, GCL (γ-GCS) (80,81). This increase in intracellular GSH could be another mechanism by which H2S indirectly participates in protection from oxidative stress.

**CHEMICAL BIOLOGY OF H2S**

H2S gas was discovered in 1777, by Carl Wilhelm Scheele, and was largely considered as a toxic gas for over hundred years (59). Based on toxicological studies, the permissible exposure limit of H2S is 10 ppm and 800 ppm exposure for 5 min is the lethal concentration for 50% of humans (LC50) (60,61). Much of its toxicity is owed to the fact that H2S is known to inhibit mitochondrial respiration and oxidative phosphorylation (46). This is further exemplified by the observations that H2S induces a state of “suspended animation” with consequent lowering of metabolic rate and body temperature in mice (61).

The standard two electron redox potential of H2S/S0 couple, −0.23 V (noted +0.140 value in acidic condition) at pH 7 (versus the standard hydrogen electrode) is comparable to that of major cellular antioxidant buffers, glutathione disulfide/glutathione (E° = −0.262 V) and cystine/cysteine (E° = −0.245 V) redox potentials (62–65). While the physiological concentration of H2S is a matter of ongoing controversy, it seems that low nM concentrations are most likely (66). Only in case of aorta, the reported concentration of free H2S is ~20–100 fold higher than that of other tissues (67). Interestingly, the flux of sulfur into H2S is comparable to that of GSH, indicating that the low nM levels are maintained as a consequence of higher sulfide clearance rate (59,68). The low steady-state concentration of H2S than GSH (~10 mM) precludes its involvement in counteracting oxidative stress by acting as an antioxidant buffer (46).

Alternatively, it is proposed that H2S can modulate intracellular redox signaling by modifying cysteine thiols of the various cellular proteins (S-persulfidation) coordinating redox homeostasis. However, since a direct reaction of H2S with thiols is unlikely, the mechanism by which persulfides are formed intracellularly is poorly understood (69,70).

H2S is lipophilic and is known to permeate freely through biological membranes without any assistance from membrane channels (lipid bilayer permeability PM ≥ 0.5 ± 0.4 cm/s) (71). Being a weak acid, it dissociates immediately and equilibrates with its anion HS− and S2− in aqueous solution as shown in Eq. (1).

\[
H_2S \rightleftharpoons H^+ + HS^- \quad K_1 \quad 2H^+ + S^{2-} \quad (1)
\]

The pK_a1 for H2S dissociation ranges from 6.97 to 7.06 at 25 °C, while pK_a2 is estimated to be between 12.20 to 15.00 at 25 °C (72). Based on these values it is calculated that the ratio of H2S:H2S is 3:1 at physiological pH of 7.4 (72). Nevertheless, total intracellular H2S levels is referred to as total free sulfide pool (i.e., H2S + HS− + S2−). Based on its chemical features, H2S can influence cellular redox physiology via four mechanisms: (1) scavenging of ROS and RNS, (2) reaction with metal centres, (3) modulation of cellular respiration, and (4) reaction with protein cysteine thiols to generate persulfides (S-persulfidation- an oxidative posttranslational modification [oxPTM]) (69,73). These mechanisms are described in the following section.

**H2S as a Free Radical Scavenger**

H2S acts as a cytoprotective molecule and has the ability to directly scavenge free radical species (74). Owing to its nucleophilic properties, H2S has been shown to react with oxygen (O2), ROS, peroxynitrite (ONOOH/ONOO−), and hypochlorite (HOCl/OCl−) (65,75). The apparent second-order rate constants of H2S with various oxidants have been summarized in Table 1. While these studies indicate a direct scavenging of oxidants by H2S in vitro, the low concentrations of H2S (10 nM to 3 μM) compared to other antioxidants (GSH; 1–10 mM) in vivo raised substantial concerns about its role in remediating ROS/RNS under biologically relevant conditions (66,76–79). Alternatively, H2S has been shown to increase GSH production by enhancing the inward transport of cystine and inducing the expression of GSH-biosynthetic enzyme, GCL (γ-GCS) (80,81). This increase in intracellular GSH could be another mechanism by which H2S indirectly participates in protection from oxidative stress.

**Reaction with Metal Centers**

The interaction of H2S with metals falls into two categories: (i) electron-transfer reaction and (ii) coordinate complex formation (65). In the first category, complete electron transfer occurs between the sulfide species and the metal, whereas coordinate complex formation involves binding of the sulfur species to the metal ligand (65). These reactions are predicted on the basis of chemical properties of H2S to act as a nucleophile. Interestingly, a wine-like model was used to study the reaction mechanism of metals with H2S. Sulfidic off-odors encountered during wine production are due to the presence of H2S and low-molecular-weight thiols (82). These off-odors are usually removed in a process called Cu fining, wherein Cu (II) is added to selectively and rapidly form ~1:4:1 H2S/Cu and ~2:1 thiol/Cu complexes, resulting in oxidation of H2S and reduction of Cu (II) to Cu (I) (82). The CuS precipitate formed is then subsequently removed from the wine by racking and/or filtration (82).
In a biological setup, interaction of H2S with the mitochondrial heme protein-cytochrome C oxidase (CcO) is extensively studied. It has been demonstrated that high concentrations of H2S competitively binds to CcO, resulting in inhibition of O2 binding (83–85). H2S interacts with CcO through the O2-binding copper (CuB/heme (a3) iron binuclear site in oxidized state (Cu2+/Fe3+) and reduces the enzyme (86). The K1 for this reaction is 0.2 μM with purified CcO (86). Most of the studies demonstrating inhibitory effect of H2S on respiration via interaction with CcO were done using very high/nonphysiological concentrations of H2S. However, it was shown that the liver mitochondria of H2S treated rats show biphasic respiration profile (87). Low concentrations (0.1–3.0 μM) of H2S induces respiration whereas higher concentrations (30–100 μM) inhibit it (87). At lower concentrations, H2S acts as a mitochondrial electron donor and stimulates electron transport chain (87,88).

Other than CcO, H2S is known to covalently modify ferryl/peroxo heme within hemoglobin and myoglobin resulting in the formation of green colored sulfhemoglobin and sulfmyoglobin species, both of which are indicators of H2S poisoning (89). Additionally, H2S can react with nonheme iron present in iron-sulfur cluster containing proteins to generate insoluble precipitates (65). Lastly, H2S is reported to react with a copper-containing protein (Cu–Zn–SOD) (90). This reaction involves copper-catalysed reduction of O2 to H2O2 and oxidation of H2S to S0 (90).

**H2S and Cellular Bioenergetics**

The effects of H2S on cellular bioenergetics are largely derived from examining mitochondrial function. The effect of H2S on mitochondria is complex, exhibiting two opposing effects; inhibition and stimulation of mitochondrial bioenergetics (88). Oxidation of H2S by mitochondrial inner membrane localized Sulfide-Quinone oxidoreductase (SQO) leads to transfer of electron from H2S to ubiquinone and increases the flux of electron transport to mitochondrial respiratory complex III and IV, thereby leading to enhanced oxygen consumption and cellular respiration (91,92).

Recently, it was demonstrated that H2S has a biphasic effect on cellular oxygen consumption/mitochondrial electron transport (87,88). These investigators creatively adapted Seahorse XF technology to precisely measure dynamic changes in mitochondrial bioenergetics in real-time in response to a gradient of H2S. Interestingly, treatment of isolated mitochondria with low H2S concentrations, NaHS (<1 μM) enhances mitochondrial oxygen consumption rate (OCR), ATP turnover rate and leads to increased maximal respiratory capacity (87). In contrast, treatment with high concentrations of NaHS (30–300 μM) causes reduced mitochondrial OCR and ATP generation, which is consistent with previous studies showing the inhibitory effect of H2S on mitochondrial respiration when present at high concentrations (84,93). The low endogenous concentrations of H2S in mitochondria is primarily maintained by mitochondrial localized 3-Mercaptopyruvate sulfurtransferase (3-MST) (87). Supplementation of isolated mitochondria with 3-mercaptoppyruvate (3-MP) leads to enhanced H2S production by 3-MST pathway and induces mitochondrial bioenergetic parameters (87). Subsequently, genetic silencing of 3-MST leads to reduced basal OCR and due to absence of 3-MST there is no stimulatory effect of 3-MP on mitochondrial bioenergetic parameters (87).

Furthermore, it was found that the stimulatory effect of 3-MP on mitochondrial bioenergetic parameters was absent in mitochondria isolated from aged mice as compared to young mice (88). Moreover, exposure of H2S was shown to cause physiological alterations which enhanced thermotolerance and life span of Caenorhabditis elegans (94). The other two PLP-dependent cytosolic enzymes CBS and CSE also maintain endogenous H2S levels. Under stress condition, CSE is known to translocate into mitochondria and stimulate mitochondrial H2S generation. This subsequently results in an increased ATP generation and resistance to hypoxia (95). Furthermore, elevated endogenous H2S in colon cancer cells have been shown to regulate cell migration and invasion (96). Additionally, pharmacological inhibition of H2S production diminished the growth of cancer cells by suppressing basal respiration, ATP production, spare respiratory capacity, and glycolysis (96,97).

**Protein Persulfidation as a Mechanism of H2S Mediated Signaling**

H2S exerts its signaling property via oxidative posttranslational modification (oxPTM) of cysteine residues, called S-persulfidation (98–100). Persulfidation (R–SSH) is the oxidation of thiol (–SH) from ~2 to −1 oxidation state (101). Being a reductant, H2S/HS− can carry out the persulfidation reaction only when one of the reagents (−SH group of proteins or H2S) is in the oxidized form (101). The proposed reactions for the formation of S-persulfide bond by the nucleophilic attack of HS− anion on reversibly oxidized protein −SH group is shown in (Fig. 4).
Recently, it was reported that persulfidation protects proteins from oxidative stress-induced damage and the over oxidized persulfidated cysteine sulfonic acid (P–SSO₃⁻) can be reversed to thiol (−SH) by the depersulfidase activity of thioredoxin (102). Thus, persulfidation can act as a protective mechanism against oxidative stress-induced protein damage (102). Using LC–MS-based techniques, S-persulfidation of proteins involved in fatty acids and carbohydrate metabolism, cellular response to stress, cell redox homeostasis, translation, and cell cycle were identified (73). Similarly, H₂S was shown to modify cysteines in about 10–25% of liver proteins including actin, tubulin, and glyceraldehyde-3-phosphate (GAPDH) by persulfidation under physiological conditions and modulate their activities (103). Several key host transcription factors, Nrf2 and NF-kB, are directly targeted by H₂S via S-persulfidation (98). Apart from eukaryotic systems, a recent study, for the first time, assessed widespread S-persulfidation of proteins in *Staphylococcus aureus* (*S. aureus*) (104). More importantly, H₂S exposure increased the level of S-persulfidation, whereas mutations in transsulfuration pathways (cysM and metB) had an opposite effect (104). Lastly, this study revealed extensive persulfidation of transcription factors involved in virulence regulation (SarA family) and interaction with host immune response (superantigen-like proteins [SSLs]) (104).

**DETECTION OF H₂S AND PROTEIN S-PERSULFIDATION**

**Tools for Detection and Quantitation of H₂S**

To achieve a better understanding of the physiological effects of H₂S, it is imperative to determine the levels of this gasotransmitter in its free and other biological forms. This becomes especially important as the role of H₂S as a protectant versus poison has been contended throughout the course of its study as a biological effector molecule. The suitability of any detection method relies heavily on three pertinent aspects namely sensitivity, reproducibility and experimental ease. A multitude of detection methodologies are available for qualitative and quantitative measurement of H₂S levels. While this may seem advantageous at first glance, it has led to a huge variability in the reported levels of “bioavailable” H₂S throughout literature. Furthermore, additional factors influencing detection arise due to experimental aspects like biological sample (tissue, sera, and cells), pH, and oxygen. The latter have been demonstrated to exert a direct effect on the stability of H₂S (105).

The detection methods range from simple colorimetric assays to techniques like gas chromatography (GC), High pressure liquid chromatography (HPLC), electrochemical, polarographic, fluorescent, and recently developed nanotechnology-based systems. Some of these techniques are summarized in Table 2. Although these methods have been widely used for biological H₂S detection, they are not devoid of pitfalls. The issues of sensitivity, invasiveness, artefactual readout, half-life, stability, permeability, lack of spatio-temporal insight, and cumbersome experimental setup cannot be overlooked. Therefore, the reporting and interpretation of experimental data becomes immensely influenced by the method that a researcher chooses to adopt. Many authors have argued that for a dynamic gaseous effector molecule like H₂S, the absolute numbers in terms of concentration may actually not matter as much as the determination of the qualitative trend of its rise and fall under different physiological conditions. This may, however, not hold true for pathophysiological conditions wherein accurate determination of H₂S levels may become indispensable for diagnosis of certain diseases.

Apart from the aforementioned techniques, recent studies using H₂S sensitive fluorescent proteins as genetically encoded biosensors have garnered immense interest. GFP molecule has been reengineered to contain the unnatural amino acid *p*-azidophenylalanine (*p*AzF). This azido group can be reduced specifically by H₂S imparting selectivity to the GFP molecule, now termed as hsGFP. An orthogonal tRNA-tRNA synthetase system from *E. coli* was used for the selective incorporation of *p*AzF into hsGFP molecule in response to the Amber (TAG) codon. In presence of both H₂S and *p*AzF, the chromophore *p*-azidobenzyldieneimidazolidone of hsGFP is converted to *p*-aminobenzyldieneimidazolidone with fluorescence excitation and emission maxima at 454 nm and 500 nm, respectively. This genetically encoded probe has been used in mammalian cell line HEK293T for the detection of intracellular H₂S. Such a genetically encoded biosensor is noninvasive and can reflect the dynamic nature of the turnover of H₂S inside cells (128). Furthermore, such sensors can be targeted to specific cellular locations. Taken together, a genetically encoded biosensor appears to be a promising tool to study the levels of H₂S under physiological conditions.

**Tools for Detection of Protein S-Persulfidation**

In addition to H₂S, the detection of the post translational modification caused by it is also of the utmost importance to fully appreciate the functional relevance of this gasotransmitter. Protein persulfidation was identified to be the mechanism by which H₂S exerts its signaling function (103). Detection of this modification, however, poses a significant challenge as the persulfide group exhibits similar reactivity to free thiols (129). Table 3 summarizes some of the widely used methods for the detection of intracellular protein persulfidation levels.

**ROLE OF H₂S IN BACTERIAL PHYSIOLOGY**

While the initial co-culture experiments described earlier provided a valuable clue with regard to potential of H₂S in protecting bacteria from toxic compounds, in depth examination of these findings was never attempted (42,43). It was only in 2011 that a study highlighted the importance of H₂S in protecting bacteria from antibiotics and oxidative stress (44). In this context, H₂S has been termed as a “double edged sword” mitigating not only the effects of antibiotics but also the resulting oxidative stress caused by them. To ascertain the role of H₂S...
in *E. coli*, the authors compared wild type and 3-MST deficient *E. coli* by a phenotypic microarray. While these strains showed no difference with respect to growth defects in vitro, the 3-MST deficient strain became highly susceptible to structurally and functionally different classes of antibiotics. Similar results were obtained for CBS/CSE deficient strains of *P. aeruginosa*, *S. aureus*, and *B. anthracis*, establishing the protective role of *H*₂*S* across gram negative and gram-positive bacteria. Overexpression of 3-MST led to enhanced protection against spectinomycin whereas chemical inhibition on 3-MST, CBS, and CSE rendered them highly susceptible to a variety of antibiotics. NaHS, an *H*₂*S* donor chemically complemented these mutant strains establishing the role of endogenously generated *H*₂*S* as a protective mechanism against antibiotics.

Several studies have shown that a wide range of antibiotics exert killing by triggering ROS generation in addition to inhibiting the function of their primary targets (135,136). Antibiotics have been shown to stimulate respiration, which increases generation of toxic hydroxyl radicals via Fe²⁺-catalysed Fenton reaction (137). Consistent with these observations the authors have shown that pretreatment with Fe-chelator (dipyridyl) or ROS scavenger (thiourea) induces gentamycin resistance to both the wild type and *H*₂*S* deficient strains of *E. coli*. Interestingly, a similar degree of protection from

| Detection method        | Description                                                                                                                                                                                                 | References                                      |
|-------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| Colorimetric            | *H*₂*S* reacts with metal salts like lead acetate, bismuth chloride, silver nitrate to form the lead sulfide which can be detected and quantified using UV-VIS spectroscopy.                                      | (106–108)                                      |
|                         | “Zinc trap” method in which zinc acetate reacts with *H*₂*S* forming zinc sulfide with subsequent acidification with *N*, *N*-dimethyl phenylenediamine. The product can be detected and quantified using UV-VIS spectroscopy.               | (109,110)                                      |
| Chromatographic         | Gas chromatography has been combined with flame photometric detectors, ion chromatography, silver particle trapping, and chemiluminescent detectors.                                                                 | (20,67,111–114)                                |
|                         | HPLC of sulfide derivatized with monobromobimane, dibromobimane, *p*-phenylenediamine, and Fe³⁺. Reverse Phase HPLC of methylene blue formed by the zinc trap assay.                                        | (115–118)                                      |
| Electrochemical         | Sulfide ion specific electrode measures *S*²⁻ form of sulfide which requires alkaline environment using Ag/Ag₂*S* electrodes.                                                                               | (119,120)                                      |
|                         | Polarographic real time measurement of *H*₂*S* using a polarographic oxygen sensor as anode and platinum wire as cathode and alkaline K₃Fe(CN)₆ as electrolyte. An *H*₂*S* permeable membrane allows diffusion of *H*₂*S* into the electrolyte solution reducing it. The electrolyte subsequently gets re-oxidized on the surface of the platinum electrode to produce a current proportional to *H*₂*S* concentration. | (121,122)                                      |
| Fluorescent sensors     | All fluorescent probes consist of a fluorescence signal transducer and the fluorescence modulator. The transducer is a suitable fluorescent moiety while the modulator is chosen based on the chemical nature of *H*₂*S* and physiologically permissible reaction kinetics. Fluorescent moieties like rhodamine, BODIPY, dansyl, 7-hydroxy-4-methylcoumarin, naphthilamide, cyanine, etc, have been used as transducers. The modulators have been designed based on the selective reduction of nitro groups to amines and azide groups to amines by *H*₂*S*, thiolysis, addition and cyclization reactions based on the nucleophilic nature of *H*₂*S* and copper sulfide precipitation resulting from the affinity of *H*₂*S* for copper. Others include selenium-based probes for reversible detection of *H*₂*S*. Probes have been designed based on the selenide-selenoxide redox reaction of many selenoenzymes. These probes can monitor redox cycling between *H*₂*S* and ROS. | (123)                                           |
| Nanotechnology-based sensors | Single walled carbon nanotube networks, gold nanoclusters, nanorods, nanocomposites of FAM DNA etched on the surface of silver nanoparticles have been employed for the detection of *H*₂*S*.                                   | (124–127)                                      |
gentamycin was observed when the cells were treated with NaHS. Additionally, all the H₂S deficient mutant strains exhibited severe susceptibility to H₂O₂ which was mitigated when they were pretreated with NaHS. DNA damage is one of the direct consequences of oxidative stress generated by antibiotics (138,139). On treatment with sublethal levels of ampicillin, which is known to cause oxidative stress, 3-MST deficient strain of E. coli showed tell-tale signs of DNA damage. Overexpression of 3-MST and pretreatment with NaHS ameliorated this damage. Additionally, the antioxidant effect of H₂S was also shown to be in part due to the stimulation of antioxidant enzymes such as catalase and superoxide dismutase (SOD). Consistent with this, the rate of degradation of H₂O₂ was significantly greater in the crude cell lysates of wild type as compared to 3-MST deficient E. coli. However, the precise biochemical mechanism by which H₂S modulates the activity of antioxidants was left unaddressed. Recently, direct sequestration of Fe²⁺ ions by H₂S has been shown to counteract
...H2O2 interacts with cysteine leading to its depletion and the expression of TcyP, a cystine importer. During oxidative stress, a series of bacteria specific H2S donors to explain MST. More recently, using chemical-biology approaches, we developed a functional association between H2S-directed cytoprotection and alternate mode of cellular respiration catalysed by cytochrome bd oxidase (CydB). H2S, due to its strong affinity for metals such as copper, is known to inhibit copper-heme containing cytochrome bo oxidase (CyoA). Under these conditions, the respiration proceeds via a less energy efficient CydB. In agreement with this, treatment of the cells with 1C led to a downregulation of cyoA transcript, whereas the transcripts of alternate respiratory oxidases such as cydB and appY were either maintained or enhanced, respectively. This realignment of respiratory oxidases mimics the expression profile of E. coli grown under respiratory arrest conditions (e.g., hypoxia), implicating H2S in respiration inhibition and metabolic slow down. In contrast, ampicillin treatment enhanced the expression of the energy efficient cyoA and repressed cydB, consistent with the reported hyperactivation of electron transport chain by bactericidal antibiotics (144). However, pretreatment of the cells with 1C reversed the influence of ampicillin on cyoA and cydB transcripts. In agreement with these findings, the cyoA mutant pretreated with 1C remained protected from ampicillin toxicity, whereas 1C-derived H2S remained completely ineffective in protecting cydB mutant. The cydB from E. coli has also been shown to reduce H2O2 by acting as catalase and quinol peroxidase (145). Therefore, sustenance of cydB expression by H2S can potentiate antibiotic tolerance by bolstering the bacterial antioxidant capacity. Altogether, these observations put forth a central role of alternate respiration and oxidant mitigating mechanisms in resisting the activity of antibiotics by H2S.

The association of H2S and drug resistance is not new. Nearly 40 years ago, several studies have reported the presence of plasmid-borne genetic elements enhancing both basal H2S production and antibiotic resistance in multidrug-resistant strains of E. coli isolated from patients suffering from urinary tract infection (146,147). Recently, we confirmed these findings and demonstrated that clinical strains of multidrug resistant uropathogenic E. coli possesses greater levels of endogenous H2S as compared to wild-type. Intriguingly, pretreating these strains with aspartate, a 3-MST inhibitor significantly reduced endogenous H2S levels and markedly reversed (50%) resistance toward ampicillin. More importantly, exposure to H2S donor neutralized the adverse effect of aspartate on drug resistance. Our work provided a strong pharmacological foundation for design of inhibitors of H2S biogenesis as a possible adjuvant to antibiotics. Further, it appears that targeting antioxidant enzymes and alternate respiratory complexes (Cyd/App) is likely to potentiate the killing efficiency of antibiotics.

The effect of H2S on intracellular human pathogens such as Mycobacterium tuberculosis (MtB) is largely unknown. However, a recent study aimed at identifying genetic components...
involved in protection from oxidative stress showed that H₂S donor (NaHS) can complement the defects in recycling of the major mycobacterial antioxidant, mycothiol (MSH) (148). Using a specific biosensor of mycolith mycolith redox potential (E_{MSH}; Mrx1-roGFP2) (143), authors have shown that NaHS restored steady-state MSH/MSSM ratio (basal E_{MSH}) in the mutants showing oxidative E_{GSH}. It is likely that NaHS increased mycothiol biosynthesis similar to H₂S-mediated increased biogenesis of GSH in eukaryotes. This supplementation with NaHS also enhanced the survival of the Mtb mycolith recycling mutants in both activated macrophages and animals. Thus, the contribution of endogenous H₂S biogenesis pathways in regulating oxidative stress and to decrease viral induced oxidative stress to control the activity of redox sensitive transcription factors Nf-κB, AP-1, and Sp1, which regulates HIV-1 gene transcription by binding to 5-LTR promoter (158,159). Despite these studies, for a very long time, the relation between HIV-1 and oxidative stress remained circumstantial. This was largely owing to the lack of sophisticated and sensitive tools to measure intracellular redox potential of HIV-1 infected cells during various stages of infection. To fill this knowledge gap, we exploited a noninvasive biosensor of GSH redox potential (Grx1-roGFP2; E_{GSH}) and accurately measured oxidative stress in the cytoplasm and mitochondria of HIV-1 infected monocytes (160). We demonstrated that monocytes laterally infected with HIV-1 are intrinsically resistant to oxidative stress and displayed reductive E_{GSH} (160). More importantly, we showed that a marginal oxidative shift in E_{GSH} (25 mV) triggers reactivation of HIV-1 without adversely affecting cellular physiology. Furthermore, supplementation with antioxidants such as N-acetylcysteine kept HIV-1 in a silent state by preventing an oxidative shift in E_{GSH} required for viral activation (160). Lastly, global expression analysis revealed that pathways associated with redox metabolism are significantly affected during HIV-1 latency and reactivation (Fig. 6) (160). Taken together, these results highlight the central role of host redox physiology in modulating HIV-1 life cycle.

Oxidative stress has been linked to vast group of etiological agents that cause acute and chronic diseases such as infection with viruses, bacteria, and parasites (149). Viral and bacterial infections in particular have been linked to induce ROS/RNS production, alteration in metabolic pathways, and leading to several disease associated complications (149,150). However, this field still lacks mechanistic insight. The impact of these infectious agents on host redox physiology and how this could be targeted for therapeutic benefits remains a challenging area of research.

In case of viral infections, induction of oxidative stress inside host is a prerequisite for successful infection and long term viral replication. RNA viruses such as influenza and paramyxovirus infection generates ROI via activation of monocytes and polymorphonuclear leukocytes (149). A study indicates that the oxidative state of the host cells provides an environment permissive for viral replication (151). Using mice models, it has been shown that influenza A (RNA virus) infection creates redox imbalance by decreasing the levels of GSH, vitamins C, and vitamin E (152). Similarly, a transgenic mouse model of hepatitis B virus (DNA virus) was exploited to show that this virus induces ROS and leads to hepatocarcinogenesis and oxidative DNA damage during chronic necroinflammatory conditions (153). Based on these studies, it has been proposed that antioxidant strategies can be utilized to target viral replication and to decrease viral induced oxidative stress to control pathological manifestations (154).

The role of oxidative stress has been extensively studied in retroviruses such as HIV-1 (Human Immunodeficiency Virus). Studies have shown that HIV-1 replication induces ROS generation and decreases cellular antioxidants such as GSH and Trx, and modulates immunopathogenesis during AIDS progression (155,156). Plasma and peripheral blood mononuclear cells (PBMCs) of AIDS patients show reduction in concentration of other major antioxidants like cysteine, methionine, vitamins C and E, along with elevated levels of lipid peroxidation products (157). At molecular level, ROS has been shown to induce the activity of redox sensitive transcription factors Nf-κB, AP-1, and Sp1, which regulates HIV-1 gene transcription by binding to 5-LTR promoter (158,159). Despite these studies, for a very long time, the relation between HIV-1 and oxidative stress remained circumstantial. This was largely owing to the lack of sophisticated and sensitive tools to measure intracellular redox potential of HIV-1 infected cells during various stages of infection. To fill this knowledge gap, we exploited a noninvasive biosensor of GSH redox potential (Grx1-roGFP2; E_{GSH}) and accurately measured oxidative stress in the cytoplasm and mitochondria of HIV-1 infected monocytes (160). We demonstrated that monocytes laterally infected with HIV-1 are intrinsically resistant to oxidative stress and displayed reductive E_{GSH} (160). More importantly, we showed that a marginal oxidative shift in E_{GSH} (25 mV) triggers reactivation of HIV-1 without adversely affecting cellular physiology. Furthermore, supplementation with antioxidants such as N-acetylcysteine kept HIV-1 in a silent state by preventing an oxidative shift in E_{GSH} required for viral activation (160). Lastly, global expression analysis revealed that pathways associated with redox metabolism are significantly affected during HIV-1 latency and reactivation (Fig. 6) (160). Taken together, these results highlight the central role of host redox physiology in modulating HIV-1 life cycle.

At molecular level HIV-1 infection or exposure to HIV-1 related proteins downregulates the Nuclear factor-erythroid-2 p45 related factor 2-Antioxidant Response Element (Nrf2/ARE) pathway, leading to reduced expression of antioxidant genes (161,162). Nrf2 is a constitutive transcription factor and master regulator of the antioxidant response (163,164). Nrf2 is inhibited in cytosol by Kelch-like ECH-associated protein-1 (Keap1), which is a redox-sensitive ubiquitin ligase substrate adaptor leading to ubiquitination and degradation of Nrf2 (165). Interestingly, Nrf2 inducer (Sulforaphane) has the ability to block HIV-1 infection in primary macrophages, which are the long-lived reservoirs of HIV-1 in infected individuals (166). In this direction, sulforaphane has also been shown to enhance phagocytic activity of HIV-1 infected monocytes-derived macrophages (MDMs) and alveolar macrophages (Ams) from HIV-1 transgenic rats, thereby reducing the severity of HIV-1 related pulmonary dysfunctions (161). Nrf2 activation has also been shown to assist Marburg virus (ss RNA) (MARV), Kaposi’s sarcoma-associated herpesvirus (dsDNA) (KSHV), and Dengue virus (ss RNA) replication. Activation of Nrf2 induced by VP24 and VFlIP proteins of MARV and KSHV, respectively, leads to dysregulation of host antiviral response and modulates viral gene expression, thereby ensuring a conducive environment for infection and also promotes the survival of infected cells (167,168). Dengue infection induced oxidative stress has been shown to activate Nrf2 thereby modulating the level of oxidative stress and affecting both antiviral and cell death response (169). While these studies clearly establish a connection between oxidative stress and infection with pathogenic viruses, the contribution of H₂S in these process is poorly
understood. Interestingly, Nrf2/Keap-1 pathway could provide the missing link between H2S, redox stress and virus infections. H2S has been shown to inhibit Keap1 by persulfidation of Cys 151, which leads to translocation of Nrf2 into the nucleus and its subsequent binding to Antioxidant Response Element (ARE). This results in the induction of genes encoding antioxidant and phase II detoxifying enzymes, such as heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase-1 (NQO-1), glutamate cysteine ligase catalytic subunit (GCLC), and thioredoxin reductase-1 (TXNRD-1) (163,165). In relation to this, H2S has been shown to protect against cellular senescence and oxidative stress via S-sulfhydration of Keap1 and resulting activation of Nrf2 (165). Further experimentation on H2S biogenesis and Nrf2/ARE pathway during viral infections will provide next stage of insight in this process. The following section provides recent knowledge in this direction.

H2S AND HOST–PATHOGEN INTERACTION

Host-derived H2S has a marked effect on the outcome of bacterial and viral infections. Blocking the host transsulfuration pathway in macrophages by propargylglycine increased the viability of *Mycobacterium smegmatis*. This impairment in bacterial clearance was shown to be due to defects in the phagolysosomal fusion during infection. Treatment with *N*-acetylcysteine (NAC), which is known to increase cysteine flux through H2S biogenesis pathway, significantly increased the phagolysosomal fusion resulting in vacuolar acidification and killing of mycobacteria (170). H2S was found to inhibit the induction of an inflammatory response on infection with *Mycoplasma fermentans*. Underlying mechanism revealed that H2S inhibited the activation and nuclear translocation of a redox sensitive transcription factor.
transcription factor NF-κB, thereby diminishing the transcription of proinflammatory genes (171,172). One of the mechanisms by which H 2S affects the activity of a global transcriptional regulator, NF-κB, is by persulfidation of Cys-38 residue in the p65 subunit (131).

H 2S production by gut microbiota presents itself as an interesting line of study to explore the effect of bacteria-derived effector molecules affecting host physiology and pathophysiology. Sulfide reducing bacteria (SRBs) represent a major class of the normal gut microbiota (173). The predominant genera residing in gut are Desulfovibrio, Desulfobacter, Desulfobulbus, and Desulfotomaculum. SRBs are the major contributors of nonenzymatic H 2S produced in the human body (174). Some of the initial studies to explore the significance of gut microbiota-derived H 2S came from experiments done on germ free animals. It was observed that fecal samples of germ free mice contained half the H 2S as compared to control mice (175). In addition to this, free H 2S levels in inferior vena cava, blood plasma and in gastrointestinal tissues, were shown to be diminished in germ free mice. Apart from this, sulfane sulfur levels of plasma, adipose, and lung tissues were also found to be lower in such mice. This implicated the gut microflora as a potential source of circulating H 2S for the host (176). Recent studies have also shown that colonocytes are capable of using H 2S as an energy source (177).

Gut bacteria-derived H 2S has been shown to have both protective and detrimental effects on colonic health. Increased fecal sulfide levels have been found in patients with ulcerative colitis (UC) (178). Furthermore, it has been suggested that epithelial damage associated with UC is due to increased availability of dietary sulfate for SRBs (179). In contrast, using animal models of colitis, it was demonstrated that scavenging bacterial H 2S by bismuth did not ameliorate the symptoms of colitis (180). In fact, the condition was shown to improve on exogenous H 2S administration (181). The ability of luminal H 2S to modify secreted defensive proteins like trefoil factor 3 (TFF3) by reduction of the disulfide bond is believed to be a potential mechanism of the anti-inflammatory role of H 2S. TFF3 plays an important role in mucosal repair and regeneration (182).

Our survey of literature revealed the existence of very few reports on H 2S and viral infections. Existing studies have highlighted the role of H 2S in modulating Respiratory Syncytial Virus (RSV) (t-ss RNA virus) infection (183). RSV causes lower and upper respiratory tract infection in infants for which there is no vaccine and only limited supportive measures for treatment exist with no real benefits (184). RSV mediates its influence on the host by upregulating the expression of various NF-κB and IRF-3 dependent cytokines and chemokines (185). This leads to inflammation, cellular infiltration in lungs and other pulmonary dysfunctions. Importantly, RSV infection resulted in downregulation of expression and impaired activity of H 2S biosynthesis enzymes. As a consequence, the endogenous levels of H 2S were diminished in RSV infected cells (183). Pharmacological inhibition or genetic silencing of CSE (cystathionine gamma-lyase) enhances RSV multiplication and exacerbates disease condition, airway dysfunction, and pulmonary inflammation (186,187). Consistent with these findings, exogenous administration of H 2S reduces the secretion of viral induced chemokines and cytokine through inhibition of NF-κB mediated activation of genes encoding proinflammatory cytokines (183). H 2S treatment (using slow releasing H 2S donor GYY4137) significantly blocked RSV replication in vitro and in vivo by targeting viral assembly, release, viral spread, and replication (Fig. 6) (183,186). Moreover, H 2S treatment significantly improved clinical disease parameters and pulmonary dysfunction on RSV infection (186). Similarly, H 2S also exerts antiviral and anti-inflammatory effects on the viruses in the family of Paramyxoviridae; human metapneumovirus (hMPV) and Nipah virus (NIV) (183).

H 2S has also been shown to affect replication of highly pathogenic enveloped RNA virus from Ortho-, Filo-, Flavi-, and Bunyavirus families (Fig. 6) (188). FDA approved antiviral treatment is available for influenza virus (Orthomyxoviridae), whereas there is no vaccine or therapeutic interventions to target Ebola virus (Filoviridae), Far-eastern subtype tick-borne flavivirus (Flaviviridae), Rift valley fever virus and Crimean-Congo hemorrhagic fever virus (Bunyaviridae) (189). H 2S was shown to significantly reduce replication of all the above families of viruses (188). As explained earlier, studies confirmed that H 2S targets the transcription factor NF-κB and IRF-3 nuclear translocation to inhibit the release of viral induced proinflammatory mediators (188).

Lastly, H 2S has been recently shown to modulate Coxsackie virus B3 (CVB3) infection induced inflammatory response, which is a predominant cause of human myocarditis and ultimately leads to heart failure (190). Treatment of CVB3 infected rats with H 2S significantly resulted in downregulation of proinflammatory mediators, reduces myocardial injury, and alleviates damage of myocardial cells (191). H 2S was shown to inhibit NF-κB signaling by lowering IκB degradation leading to reduced nuclear translocation and DNA binding ability of NF-κB (191). CVB3 infection also induces MAPK signaling cascade by activating ERK1/2, p38 and JNK1/2 which are upstream signaling molecule involved in activation of NF-κB (192). H 2S treatment also showed reduced CVB3 induced activation of ERK1/2, p38, and JNK1/2 in rat myocardial cells, thereby suppressing the expression of inflammatory mediators and alleviating myocardial damage (191).

CONCLUSION
A bourgeoning body of literature stands testament to the cytoprotective role of H 2S in various organs and tissues, ameliorating a wide variety of stresses. In comparison, the importance of this gasotransmitter from the perspective of bacterial physiology and pathogenesis remains understudied. This aspect becomes increasingly important as H 2S has been shown to confer protection from both antibiotic and oxidative stresses. In today’s scenario, where drug resistance is becoming one of
the leading healthcare concerns, there is an immediate need to have a greater understanding of the role of \( \text{H}_2\text{S} \) in bacterial antibiotic resistance. Additionally, it would be worthwhile to study the importance of this molecule in intracellular bacteria, which face a hostile oxidative host environment.

Viral infections are accompanied by host physiological perturbations like alteration in redox homeostasis, inflammation, metabolism, among others. Of particular interest to us is the observation that a number of viruses have been shown to adversely affect the host \( \text{H}_2\text{S} \) biogenesis. Antiviral and anti-inflammatory effects of \( \text{H}_2\text{S} \) highlight its potential as a therapeutic molecule. It can, therefore, bolster the efficacy of the regular drug regime used for viral infections. Despite all these observations there is a dearth of knowledge in terms of molecular mechanism of these effects which could form a promising line of research. Through our review, we have highlighted some of the important studies in this field pertaining to the role of \( \text{H}_2\text{S} \) in physiology and pathogenesis of these infectious agents. Considering that the role of \( \text{H}_2\text{S} \) as a cytoprotectant appears to be conserved across phyla, the significance of this ubiquitous biological effector molecule in bacteria and viruses should be pursued with renewed interest.

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CONFLICT OF INTEREST

The authors indicate no potential conflicts of interest.

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