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FORUM REVIEW ARTICLE

# Endogenous Production of $H_2S$ in the Gastrointestinal Tract: Still in Search of a Physiologic Function

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**Abstract**

Hydrogen sulfide ($H_2S$) has long been associated with the gastrointestinal tract, especially the bacteria-derived $H_2S$ present in flatus. Along with evidence from other organ systems, the finding that gastrointestinal tissues are capable of endogenous production of $H_2S$ has led to the hypothesis that $H_2S$ is an endogenous gaseous signaling molecule. In this review, the criteria of gasotransmitters are reexamined, and evidence from the literature regarding $H_2S$ as a gaseous signaling molecule is discussed. $H_2S$ is produced enzymatically by gastrointestinal tissues, but evidence is lacking on whether $H_2S$ production is regulated. $H_2S$ causes well-defined physiologic effects in gastrointestinal tissues, but evidence for a receptor for $H_2S$ is lacking. $H_2S$ is inactivated through enzymatic oxidation, but evidence is lacking on whether manipulating $H_2S$ oxidation alters endogenous cell signaling. Remaining questions regarding the role of $H_2S$ as a gaseous signaling molecule in the gastrointestinal tract suggest that $H_2S$ currently remains a molecule in search of a physiologic function. *Antioxid. Redox Signal.* 12, 1135–1146.

A LONG-STANDING ASSOCIATION exists of hydrogen sulfide ($H_2S$) with the gastrointestinal tract. After the initial systematic description of the physical properties of $H_2S$, chemists of the late 18th and early 19th centuries, by using the intestines of dead and decaying animals, identified $H_2S$ gas as a major constituent of the process of putrefaction. Lehmann (33) described the culmination of several studies in the first half of the 19th century that documented $H_2S$ as a constituent of intestinal gas before postmortem putrefaction. The small amount of $H_2S$ in normal flatus made early chemists consider digestion in the large intestine as the same chemical process as putrefaction, which led many physicians, including Dr. John Kellogg, creator of Kellogg's Corn Flakes, to prescribe colonic cleansing or hydrotherapy to maintain, perhaps inappropriately, colonic health. At the advent of microbiology, Gayon (18) described the ability of isolated bacteria to generate $H_2S$ from albuminous material. His work ushered in a flurry of taxonomic and biochemical studies in the late 19th century and the first half of the 20th century regarding the enzymatic production of $H_2S$ by commensal bacteria.

Whereas the vast majority of early scientific studies regarding $H_2S$ and the gastrointestinal tract dealt with $H_2S$ of bacterial origin, recent work on $H_2S$ is focused on the role of endogenously produced $H_2S$ from nonbacterial sources. This recent activity is due to the emergence of $H_2S$ as an endogenous signaling molecule. A mere 13 years ago, Abe and Kimura (1) described the enzymatic mechanism of $H_2S$ production in the brain and the biologic effects of and cellular targets for $H_2S$. Their work began what was to become an exponential increase in studies related to the cell-signaling properties of $H_2S$. The disciplines of chemistry, pharmacology, molecular biology, and physiology have converged to support the hypothesis that $H_2S$ is an endogenous molecule that regulates physiologic and pathophysiologic processes. The intention of this article is to review critically the literature that supports the role of $H_2S$ as a gaseous signal molecule in the gastrointestinal tract. In some areas of this review, data from other organ systems are included to highlight areas that require attention in the gastrointestinal tract. To set the stage for this review, we first provide our views on the use of the term gasotransmitter and follow with a reevaluation of the criteria being considered for $H_2S$ as a gaseous signaling molecule.

## Gasotransmitter Criteria Revisited

The term gasotransmitter implies that the candidate gaseous molecule is synthesized and released into the extracellular spaces to transmit a signal by acting on another cell. When released from a neuron, a candidate gaseous molecule can be considered to function as a neurotransmitter, by following classic definition for a neurotransmitter (70). The

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elevation of intracellular calcium and induction of calcium waves in astrocytes and microglia in the central nervous system (CNS) by NaHS suggests that endogenous $H_2S$ functions *in vivo* as a neurotransmitter. When $H_2S$ is released from nonneuronal cell types, including intraluminal colonic sulfur-reducing bacteria, use of the term transmitter to describe the action of $H_2S$ also is appropriate. For the past several years, a substantial amount of data has been acquired to support the hypothesis that $H_2S$ can also act on targets within the cell in which it is produced. In this regard, $H_2S$ functions as an intracellular signaling molecule. In this review, we use the term gaseous signaling molecule to include both putative transmitter and intracellular signaling roles of $H_2S$.

In 2002, Rui Wang (67) set forth the following five criteria for a molecule to be classified as a gasotransmitter and included evidence demonstrating that $H_2S$ met these criteria. Gasotransmitters must

1. be small molecules of gas,
2. be freely permeable to membranes,
3. be endogenously and enzymatically generated in a regulated manner,
4. have well-defined specific functions at physiologically relevant concentrations, and
5. act at specific cellular targets.

These criteria have been very helpful to define the focus of the field of $H_2S$ biology. We propose that they can now be updated.

With regard to the first criterion, it is not necessary to constrain the size of a putative gasotransmitter. We propose removing the word "small." Although NO, CO, and $H_2S$, are freely diffusible through membranes, it is unclear why putative gasotransmitters must fulfill the second criterion. Gaseous molecules that are polar and not freely diffusible would not meet the second criterion but may still be considered gaseous signal molecules. Therefore, we propose eliminating this criterion. The third criterion, a regulated enzymatic pathway for the production of the gas, is reasonable. Although Wang's fourth criterion also is reasonable, more consideration should be given to what are physiologically relevant concentrations. In the case of $H_2S$, a number of original research articles and reviews (compiled by 71) have stated that $H_2S$ is an endogenous signal molecule, because resting tissue and plasma levels of $H_2S$ are similar to the effective concentration of exogenous NaHS. Most *in vitro* studies of the influence NaHS of on tissue function have required a tissue bath concentration of 100 $\mu M$ to alter tissue activity appreciatively. Although several studies have identified tissue concentrations in the micromolar range, these measurements have been called into question (63, 71), and a recent report indicates that the free $H_2S$ concentration in tissue (brain and liver) appears to be only $\sim$20 n$M$ (16), roughly 1/5,000 of the requisite bath concentration. Because inhalation of 2,240 ppm $H_2S$ (equivalent to 100 $\mu M$ $H_2S$) is lethal to animals, it becomes important to determine what actions of $H_2S/HS^-$ observed in *in vitro* studies reflect toxicity to tissue *versus* physiologically relevant signaling of tissue activity. Several possible explanations exist to explain how $H_2S$ could serve as a gaseous signal molecule, despite the large discrepancy between the concentrations of $H_2S$ in tissue *versus* those needed for alteration of tissue function, *in vitro*. First, diffusion of $H_2S/HS^-$ into even relatively small pieces of tissue (*i.e.*, 50 mg) is relatively slow. Most tissues have an effective means of metabolizing $H_2S$, so a gradient of $H_2S$ concentration will develop in the tissue, with only the most superficial layer having an $H_2S$ concentration equal to that of the bath, whereas cells in the interior of the tissue would be exposed to much lower, even trivial, concentrations of $H_2S$. Second, because $H_2S$ is both produced and consumed by cells, concentrations of $H_2S/HS^-$ at localized sites of release in the cell may be much higher than those observed in whole tissue. As a result, receptor-like molecules located in proximity to the release site could be exposed to high $H_2S$ concentrations, whereas the overall tissue concentrations remain low.

The complexity of relating the concentration of $H_2S$ in the bathing solution to a physiologically relevant concentration is an echo of earlier research on acetylcholine as a neurotransmitter. Although acetylcholine was discovered in the 1920s, it was not possible to measure acetylcholine levels accurately prior to 1965, and tissue levels were determined to be relatively high. Today, extremely sensitive methods are available for its detection. The level of acetylcholine in the cerebrospinal fluid of humans is $\sim$0.3 n$M$ (74), whereas the half-maximal effective concentration ($EC_{50}$) value for acetylcholine activation of $\alpha_4\beta_3$ postsynaptic nicotinic acetylcholine receptors is $\sim$75 $\mu M$ (47). Yet, the validity of acetylcholine as a neurotransmitter in the CNS is not questioned. It is safe to assume that, as in the early days of acetylcholine biology, it has not yet been possible to measure accurately the concentration of "physiologically relevant" $H_2S$. The development of fluorescent markers to determine the spatiotemporal concentrations of $H_2S$ will likely be a key advance in the future. We suggest that it is important to include within the language of the fourth criterion that physiologic effects occur on exogenous application of physiologically relevant concentrations of the candidate molecule, such that it mimics the effect of the proposed endogenous signaling pathway.

We also propose that the fifth criterion regarding specific cellular targets be modified. A drug is classified as acting at a receptor only if it can be competitively antagonized by a related molecule (26). Competitive antagonism remains a key criterion of neurotransmitters and signaling molecules, as it is the accepted method of determining that a specific receptor exists for the drug. We propose adding the concept of competitive antagonism to the fifth criterion, despite the lack of experimental evidence for this criterion being fulfilled for $H_2S$ (see later).

Finally, a key criterion in the case of $H_2S$ acting as a neurotransmitter (70) or signaling molecule that is absent from Wang's list is a mechanism for inactivation. An important aspect of the information encoded by a signal molecule, especially a neurotransmitter, is a temporal signal. Regulated control of the duration of cell signaling is as dependent on a specific mechanism of signal termination as it is on the mechanisms of regulated synthesis and release. Few signal molecules rely on passive diffusion, and thus, dilution of the signal molecule as a form of inactivation. Rather, most signal molecules use transporter uptake or enzymatic degradation to shorten the duration of the response. Uptake and degradation help protect the cells receiving the signal from overstimulation, which is often fatal to the cell, and help keep systemic concentrations of the active molecule from reaching high concentrations. We propose adding this criterion to the list and suggest that the rapid oxidation of $H_2S$ by tissues be considered in future studies of $H_2S$ physiology (see later).

We propose that the criteria for a gasotransmitter be revised such that a gaseous signaling molecule must

1. be a gas,
2. be endogenously and enzymatically generated in a regulated manner,
3. with exogenous application, cause a well-defined physiologic effect at physiologically relevant concentrations that mimics the effect of the endogenously produced $H_2S$ on tissue activity,
4. act at specific cellular targets, as demonstrated by competitive antagonism, and
5. employ a specific mechanism of inactivation.

The remainder of this review is devoted to highlighting the current evidence that $H_2S$ fulfills some of these criteria, with an emphasis on evidence from the gastrointestinal tract, and highlighting areas of research that require attention for these criteria to be met. Because the third and fourth criteria overlap, they are considered together.

## The First Criterion: $H_2S$ is a Gas

The physical properties of $H_2S$ gas are well described (4, 45). $H_2S$ exists as a gas because its boiling point is approximately $-60^{\circ}C$. Aqueous solutions absorb $H_2S$ such that, at equilibrium, $H_2S$ in the aqueous phase is $\sim 2.2$ times the concentration of the gas phase at 37°C. Increased temperatures slightly decrease the solubility of $H_2S$, but changing pressure has little effect on this equilibrium. In aqueous solution, the $pK_a$ of the dissociation of $H_2S$ to $HS^-$ is 7.02 at 25°C. Because $pH = pK_a + \log_{10}([HS^-]/[H_2S])$, the ratio of $[HS^-]/[H_2S]$ at pH 7.4 must have an antilog equal to 0.38 (7.4 − 7.02); thus, the ratio of $[HS^-]$:$[H_2S]$ is 2.4 at pH 7.4. Although measurements of intracellular pH have been quite variable, many such measurements have indicated that intracellular pH is ~7.0, which would result in roughly equal intracellular concentrations of $H_2S$ and $HS^-$. However, because the $pK_a$ of this dissociation decreases with increased temperature, the ratio of $[HS^-]$:$[H_2S]$ is higher at 37°C (12). This discussion underscores the concept that dynamic changes in temperature and pH can dramatically affect the concentration of dissolved $H_2S$ in physiologic preparations. Although $HS^-$ can dissociate to $H^+$ and sulfide ($S^{2-}$), the $pK_a$ of this dissociation is ~12; thus, negligible $S^{2-}$ exists at physiologic pH.

Although it has been suggested that the active form of $H_2S$ *in vivo* may be $HS^-$ (12), which is not a gas, the source of endogenous $HS^-$ is the dissociation of enzymatically produced $H_2S$. In the literature, the use of the term $H_2S$ usually refers to the sum of free $H_2S$ and $HS^-$, as is the case in this review. $H_2S$ is lipid soluble and not constrained by cellular membranes, but due to its partial dissociation, the lipophilic plasma membrane is less permeable to $H_2S$ than it is to NO and CO.

## The Second Criterion: Endogenous Production of $H_2S$

### *$H_2S$-producing enzymes*

**General interdisciplinary view of $H_2S$-producing enzymes.** The original demonstration of biologic production of $H_2S$ by bacteria (18) began nearly a century and half of comprehensive studies describing the biochemistry of $H_2S$ production. $H_2S$ is a constituent of intestinal gas and is the principal by-product of sulfur-reducing bacteria (desulfovibrio) in the mammalian colon. $H_2S$ concentrations in excess of 1,000 ppm have been measured in gas samples obtained from the rat cecum (59). $H_2S$ and methyl mercaptan also are produced by the flagellate bacterium *Helicobacter pylori* and may account in part for halitosis (31).

As in bacteria, $H_2S$ gas is formed naturally in vertebrates, including humans, through the activity of cystathionine $\beta$ synthase (CBS), cystathionine $\gamma$ lyase (CSE), both 2-pyridoxal-5′-phosphate (PLP) dependent, and 3-mercaptopyruvate sulfurtransferase (3MST) (23, 56, 57), a non–PLP-dependent enzyme. CBS and CSE may function as cellular redox sensors, increasing $H_2S$ generation in response to intracellular oxidant load (38). Physiologic acidification is required for 3MST activity (23).

Although several compounds are used to inhibit $H_2S$-synthesizing enzymes, a recent call was made for the development of new pharmacologic tools with improved selectivity and specificity (62). Aminooxyacetate and hydroxylamine are used to inhibit CBS. As both compounds target the PLP-binding domain of CBS, they also are effective inhibitors of other PLP-dependent enzymes, including CSE and aminotransferases. DL-Propargyglycine and $\beta$-cyano-L-alanine are used as irreversible and reversible inhibitors, respectively, of CSE and demonstrate a certain degree of specificity. Hydrogen peroxide and tetrathionate inhibit 3MST by interfering with the catalytic cysteine residue, similar to the effect these compounds have on all enzymes that use cysteines in the catalytic site. The 2- and 3-mercaptopropronic acids are uncompetitive and noncompetitive inhibitors of 3MST, respectively.

The human *CBS* gene is located on chromosome 21 (21q22.3) (30) and encodes several splice variants (19). An autosomally inherited recessive deficiency of CBS through multiple genetic polymorphisms results in homocysteinuria (29). CBS activity and hence $H_2S$ production is increased in Down syndrome (25). The structure of a truncated form of human CBS has been solved (44). The gene that encodes human CSE (*CTH*) is located on chromosome 1 (1p31.1), encoding at least two splice variants (37). Polymorphisms in the CSE gene are associated with cystathionuria (66). The structure of CSE, bound to its inhibitor propargylglycine, has been recently solved (Fig. 1) (28, 61). This advance is likely to result in the development of new and more-selective enzyme inhibitors. The gene that encodes human 3MST (*MPST*), also known as liver rhodanese and thiosulfate sulfurtransferase 2 (TST2), is located on chromosome 22 (22q11.2) and encodes at least three splice variants (6, 50). This gene should not be confused with thiosulfate sulfurtransferase (*TST*) or rhodanese, which is immediately adjacent on chromosome 22 (22q13.1) but encodes a distinct enzyme (2). Polymorphisms in 3MST may underlie mercaptolactate-cysteine disulfiduria (6).

**$H_2S$-producing enzymes in the gastrointestinal tract.** Transcripts encoding both CSE and CBS have been identified in gastrointestinal tissues in rats (13) and mice (36). Both CSE and CBS are expressed, as determined by immunoreactivity, in the mouse colonic mucosa, whereas only CSE is expressed in the external muscle layers of the colon, with the highest level of immunoreactivity in enteric neurons (36). The very low expression levels of mRNA encoding CBS in the external muscle layers, including the myenteric plexus, suggests that CSE activity predominates in the mouse colon. The absence of

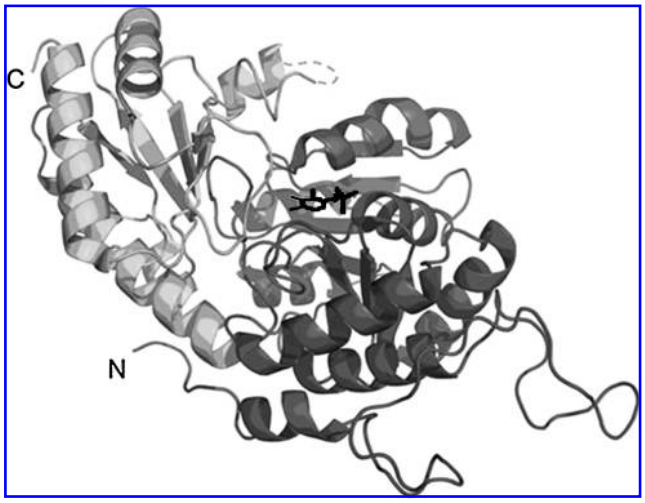


**FIG. 1. Ribbon diagram of human CSE monomer derived from x-ray crystallography data.** Pyroxidyl-5-phosphate (PLP) is shown in black, and the PLF-binding domain is shown in the darker shade of gray. The smaller domain of CSE is shown in light gray. Native CSE likely exists as a tetramer. Reproduced with permission from (61).

CBS in mouse colonic enteric neurons is at variance with a previous report that both CBS and CSE are present in guinea pig and human enteric neurons of the colon (55). This difference may reflect species differences. The use of specific inhibitors of CSE and CBS and measurement of endogenous production of $H_2S$ gas may be helpful in determining the relative contribution of the two PLP-dependent enzymes in the same organ across species.

### *$H_2S$ production and release*

General interdisciplinary view of $H_2S$ production. Historically, the presence of $H_2S$ gas has been qualitatively measured by its ability to discolor metals. The ability of metals to absorb $H_2S$ has been used until the present day to "trap" sulfide as a stable solid (58). On acidification of stable and soluble metal-sulfide, $H_2S$ gas is released and can be measured quantitatively with numerous techniques. Perhaps the most common method of $H_2S$ gas measurement is the methylene blue assay, the history and use of which is described by Jacobs and colleagues (24). $H_2S$ reacts with *N,N* -dimethyl-*p*-phenylenediamine and ferric chloride in a strongly acidic solution to produce methylene blue [3,7-bis (dimethylamino) phenazathionium chloride], which can be quantitatively measured with colorimetric or spectrophotometric analysis.

New methods are being developed to assay $H_2S$ gas production. Polarographic technology is being used to demonstrate $H_2S$ release from sulfide-containing compounds (11). Electrodes sensitive to $H_2S$ are being used in a variety of tissues, and the results support the concept that previous reports of $H_2S$ production greatly overestimate $H_2S$ levels (71). Gas chromatography remains an accurate and reliable method of measuring $H_2S$, but methods used to collect the gas sample have recently been scrutinized. $H_2S$ gas is highly labile, and samples degrade rapidly, even in sealed storage containers. Furne and colleagues (16) recently reported that when tissue was rapidly homogenized, and the gas released from the tissue was assayed immediately and directly for $H_2S$, the tissue contained only $\sim 10^{-8}$ *M* as compared with previous estimates of $10^{-5}$ *M* (35). Furne and colleagues (16) suggested that the discrepancy might arise from the addition of cysteine (up to 10 m*M*) in the tissue homogenates to stimulate $H_2S$ production, which was not added in their analysis. Cysteine in solution is capable of nonenzymatic decomposition to $H_2S$ that can be readily detected by smelling a vial of dissolved cysteine.

Several studies demonstrated that $H_2S$ production is altered by experimental manipulation. By using the enzymatic assay of acid-induced release of $H_2S$ in the presence of cysteine, $H_2S$ production *in vivo* is increased after ischemia (77) and decreased in diabetic mice (7). In cell culture, incubation of $H_2S$-producing cells with glucose (76) or the NO-releasing compound sodium nitroprusside (79) increases $H_2S$ production. It is of interest to note that because these measurements were obtained through enzymatic assays with excess cysteine, these measurements may have simply reflected increased expression of the enzymes rather than a change in their production levels. In a recent and elegant study, the constitutive production of $H_2S$ was found to be offset by mitochondrial oxidation (49). As tissue $O_2$ decreases, $H_2S$ oxidation declines, thereby increasing the cellular concentration of $H_2S$ to exert its physiologic effect.

$H_2S$ production in the gastrointestinal tract. Several groups have measured the enzymatic production of $H_2S$ in the gastrointestinal tract (13, 22, 36). Unlike $H_2S$-production assays in other regions of the body, the presence of sulfate-reducing commensal bacteria within the lumen of the gastrointestinal tract can contribute to the amount of $H_2S$ that is measured. To eliminate the contribution of luminal bacteria, we developed a method to measure quantitatively the endogenous generation and release of $H_2S$ gas in intact and living muscle layers of the mouse colon, containing the myenteric plexus dissected away from the mucosa, without contamination of luminal bacteria (36). The rate of $H_2S$ gas production and release from intact tissue averaged 0.45 pmol/min/mg tissue and was inhibited when the tissue was treated simultaneously with CSE and CBS inhibitors. The level of nonbacterial $H_2S$ production in intact tissue is at least an order of magnitude lower than the level of $H_2S$ production in the same tissue after it was homogenized (36) and considerably lower than previous reports for homogenized tissues (13, 22). These observations suggest that either homogenization disrupts the normal proximity of the enzymes that produce $H_2S$ gas to the enzymes responsible for its degradations or that the relatively high levels of $H_2S$ previously reported were not of free $H_2S$ gas but instead of $H_2S$ released from acid-labile and bound cytoplasmic stores (23), or both. As described earlier, current methods being used to measure $H_2S$ rely on the presence of exogenous cysteine. By using the method developed by Furne and colleagues (16) without the addition of cysteine, we recently determined that mouse colonic muscle tissue, obtained aseptically without luminal bacteria or the addition of cysteine, contains $\sim 60$ n*M* $H_2S$ (Fig. 2).

Because CSE is highly expressed in enteric neurons, we conducted experiments to test whether $H_2S$ production and release could be altered by electrical field stimulation (EFS) by using standard protocols for nerve stimulation and our method of measuring $H_2S$ production from living tissue (36) (Fig. 3). EFS delivered by bipolar platinum-ring electrodes

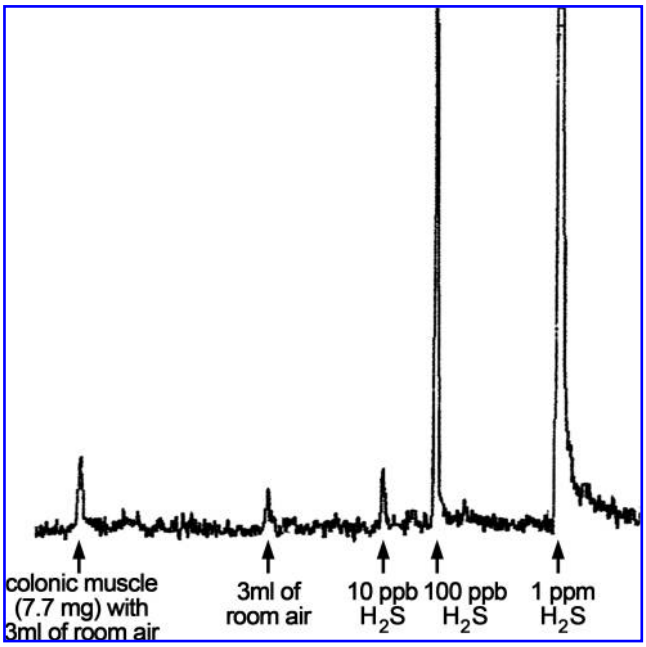


**FIG. 2. Facsimile of representative elution peaks of $H_2S$ by using gas chromatography and sulfur fluorescence detection, demonstrating that the external muscle layers of the colon contain $H_2S$ without supplementation with cysteine or other stimulation.** Whereas room air contained ~8 ppb $H_2S$, 3 ml of room air mixed vigorously with colonic tissue contained ~13 ppb $H_2S$. With the mass of the tissue and assumed equilibrations between dissolved and gaseous $H_2S$ and $H_2S$ and $HS^-$, the calculated concentration of $H_2S$ in the tissue was ~60 n*M*.

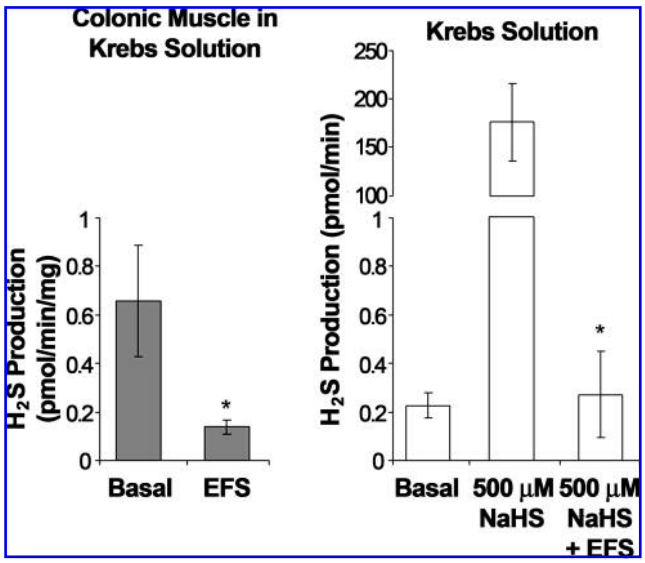


**FIG. 3. With the methods of Linden and colleagues (36), $H_2S$ produced in 1 ml of normal Kreb's solution containing 10 m*M* cysteine and either intact living colonic muscle (14 to 20 mg) (left) or 500 $\mu M$ NaHS (right) were subjected to electrical field stimulation (EFS) by bipolar platinum ring electrodes separated by 0.5 cm (trains of 0.5-ms pulses, 10 Hz for 30 s, every 5 min for 30 min).** EFS likely oxidized all $H_2S$ in solution to sulfate at the anode. Data are expressed as mean ± SEM; $n = 3$; *$p < 0.05$; ANOVA or *t* test compared with unstimulated.

eliminated $H_2S$ gas in the samples. However, subsequent experiments using a solution of NaHS without tissue revealed that $H_2S$ was eliminated in an electrochemical manner. In retrospect, the electrochemical properties of $H_2S$ are well known, as metal anodes effectively oxidize $H_2S$ to sulfate (45), which forms the basis for the electrochemical detection of $H_2S$ (71). We are currently investigating other mechanisms of nerve stimulation to determine whether $H_2S$ gas production and release in the colon wall can be regulated by enteric neuronal activity.

A strong potential source of acute $H_2S$ regulation is the dynamic availability of free cysteine. Cysteine is "stored" in proteins, sulfomucins, and in glutathione, such that cytosolic levels are normally quite low. Cysteine availability appears to be responsible for changes in the levels of $H_2S$ in flatus. Lack of suitable substrate in the colonic chyme sulfomucins can limit the amount of $H_2S$ produced (59). The rate-limiting step in $H_2S$ production may be the dynamic and regulated release of free cysteine.

Summary of the second criterion. Collectively, the literature supports the view that $H_2S$ is produced endogenously in the gastrointestinal tract in partial fulfillment of the second criterion. Potential regulation of endogenous $H_2S$ production remains a question that requires further investigation. Future studies that test the dynamic regulation of $H_2S$ production by intestinal tissues are likely to reveal mechanisms that can be applied to other organ systems.

## The Third and Fourth Criteria: Physiologic Effects of $H_2S$ at Specific Cellular Targets

### *General interdisciplinary view of the action of $H_2S$*

The well-described activation of soluble guanylyl cyclase (sGC) by epithelium-derived relaxing factor (EDRF), and the subsequent demonstration that NO reversibly and competitively binds the heme moiety of sGC led to general acceptance of NO as a gasotransmitter because cytosolic sGC acts like a classic membrane-bound receptor for the endogenous ligand NO (5). Unlike NO, no well-described orphan receptor is waiting for $H_2S$ to fulfill the role of an endogenous ligand. The search for potential receptors for $H_2S$ has used pharmacologic approaches that implicate potential interacting proteins. However, specific receptors for $H_2S$ have not been identified. Cytochrome *c* oxidase, the molecular target for $H_2S$-mediated toxicity, remains the only protein demonstrating $H_2S$ binding in a reversible fashion (48). The lack of demonstrable $H_2S$-binding sites and competitive antagonism (a criterion of a gaseous signal molecule set forth in a previous section of this review) has led to much speculation regarding the identity of receptors for $H_2S$, but to little direct evidence for ligand–receptor interaction.

Putative molecular targets appear to be both tissue and species dependent. $H_2S$ potentiates NMDA receptors during repetitive nerve stimulation (1) through cAMP-dependent pathways, opens $K_{ATP}$ channels (17, 80), voltage-gated potassium channels (17), apamin-sensitive potassium channels (17), $Ca_V3.2$-T-type channels, and chloride channels expressed in somatic and peripheral primary afferent pro-nociceptive neurons (43), TRPV1 channels (55), and TRPA1 receptors (39).

The high affinity of sulfur to react with divalent metallic cations may be the underlying mechanism by which $H_2S$ activates $Ca_V3.2$-T-type channels. It has been proposed that $H_2S$ chelates extracellular $Zn^{2+}$ normally bound to the channel, thereby opening the channel (40). Opening of $K_{ATP}$ channels in vascular smooth muscle may be due to intracellular acidification, which in turn is due to activation of the $Cl^-/HCO_3^-$ exchanger (32).

Recent evidence suggests that the thiol residues of many of the previously mentioned targets are sulfhydrated by $H_2S$ (46). Sulfhydration appears to be a major posttranslational modification of actin, GAPDH, tubulin, and the cysteine residue of $K_{ATP}$ (46). Although sulfhydration may be an attractive explanation of the biologic effects of $H_2S$, some facts suggest that this mechanism lacks the specificity and reversibility needed to be considered receptor binding. Sulfhydration is a covalent modification of existing thiol residues. Although covalent modification of cysteine residues has been proposed as the activation mechanism of TRPA1 receptors (39), this mechanism is not well characterized and deviates from well-characterized reversible ligand–receptor interactions (26). To terminate receptor activation caused by sulfhydration, desulfhydration through currently unknown mechanisms would have to be part of the process. Ligand binding of numerous neurotransmitter receptors is altered by the redox manipulation of extracellular cysteine residues (for example: 60 and 65) suggesting that $H_2S$ may act on these receptors as well. The potential modification of a wide variety of membrane proteins through sulfhydration would suggest that sulfhydration is a nonselective mechanism of action, which would argue against the existence of specific $H_2S$ receptors activated by sulfhydration. If this mechanism remains the only mechanism of action of $H_2S$, its promiscuity, in the strict sense, would argue against $H_2S$ qualifying as a gaseous signal molecule.

### *The action of $H_2S$ in the gastrointestinal tract*

**$H_2S$ acts on nervous tissue.** In the gastrointestinal tract, exogenous application of NaHS has tetrodotoxin-sensitive, prosecretory effects in the guinea pig colon through sensory nerve endings that send collaterals to the mucosa or to secretomotor neurons, which cause secretion, and excites some neurons in the guinea pig myenteric plexus (55). Luminal application of NaHS in the colon increases cFos staining in the spinal cord and enhances nociceptive reflexes, suggesting that $H_2S$ activates primary afferent neurons that innervate the colon (43). This effect, somewhat surprising, given the rapid conversion of luminal $H_2S$ to thiosulfate (34), appears to be mediated by activation of T-type voltage-gated calcium channels (43). In recent experiments done in our laboratory, we found that NaHS (100 $\mu M$) *in vitro* facilitates synaptic transmission in mouse sympathetic ganglion neurons that innervate the colon (Fig. 4).

**$H_2S$ acts on smooth muscle.** With few exceptions, exogenously applied NaHS inhibits gastric and intestinal motility. Circular muscle from guinea pig ileum precontracted with acetylcholine is relaxed by NaHS in a concentration-dependent manner (22). Application of NaHS reduces spontaneous and acetylcholine-induced contractions in the rabbit and rat ileum, effects not blocked by glibenclamide, suggesting that $K_{ATP}$ channels are not involved (64). NaHS also has an inhibitory effect on muscle contraction in the human, rat, and mouse colon, as well as in the mouse jejunum (17). The inhibitory effect of NaHS in the mouse is unaffected by tetrodotoxin, NOS inhibitors, and PPADS and is retained in the TRPV1-knockout mouse, suggesting that the action of NaHS is directly on smooth muscle cells (17). The direct inhibitory effect is largely through an action on multiple potassium channels, particularly apamin-sensitive small conductance channels and glibenclamide-sensitive $K_{ATP}$ channels (17). In the mouse and human colon, the hyperpolarizing response to NaHS (1 m*M*) *in vitro* was not blocked by the $K_{ATP}$ channel blocker glibenclamide (20 $\mu M$), suggesting that the response to NaHS is not mediated through $K_{ATP}$ channels (Fig. 5).

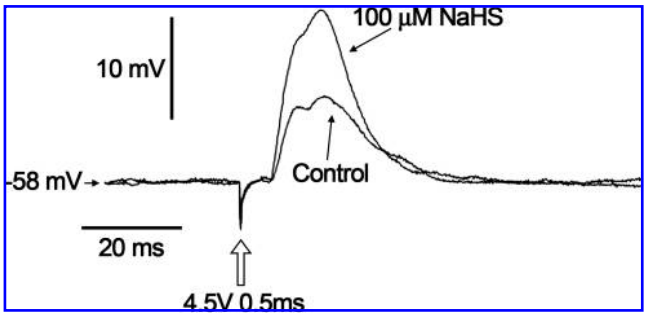


**FIG. 4. NaHS-induced facilitation of a fast excitatory postsynaptic potential in a mouse superior ganglion neuron evoked by stimulating the lesser splanchnic nerve.** Data are provided courtesy of Dr. Lei Sha.

**$H_2S$ acts on ICC.** In the gastrointestinal tract, smooth muscle function can be manipulated indirectly through the pacemaker interstitial cells of Cajal (ICCs) that are responsible for phasic fluctuations in smooth muscle membrane potential. Conflicting evidence regards the presence of CBS and CSE in ICCs. ICCs in the guinea pig colon are immunopositive for CSE but not for CBS (55), whereas in preliminary data reported in abstract form, no evidence for CSE and CBS mRNA was found in ICCs from the mouse colon (27). However, an

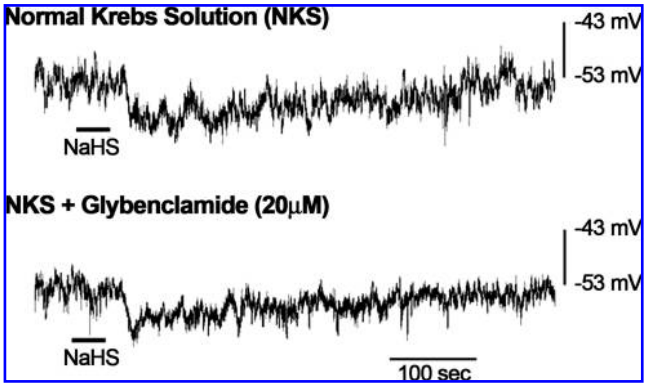


**FIG. 5. Hyperpolarization of mouse circular smooth muscle induced by NaHS (1 m*M*, applied locally with a micropipette) was not blocked by glibenclamide.** Data are provided courtesy of Dr. Lei Sha.

action of $H_2S$ on ICCs remains a possibility, irrespective of whether ICC synthesizes $H_2S$, because NaHS reduces the frequency of phasic contraction (17). Preliminary data presented in abstract form show that NaHS (100 to 300 $\mu M$) depolarizes ICCs and activates the nonselective cation channels responsible for pacemaker activity and slightly enhances spontaneous calcium waves, whereas high concentrations of NaHS (1 m$M$) activate $K_{ATP}$ channels and increase mitochondrial uptake of calcium, causing inhibition (27). None of the effects were blocked by ODQ, the adenylate cyclase inhibitor (SQ22536), or the NOS inhibitor L-NAME.

Summary of the Third and Fourth Criteria. It is clear from studies that use exogenous application of NaHS that a variety of effects in the gastrointestinal system may involve a variety of mechanisms. What is lacking is rigorous pharmacologic examination to determine the nature of the antagonism (26). Competitive antagonism demonstrated by Schild analysis remains the standard method to implicate receptor action of a potential protein. Future studies that implicate a potential receptor should use this proven pharmacologic approach to determine whether the suspected protein is indeed a receptor for $H_2S$.

### The Fifth Criterion: Inactivation of $H_2S$

#### *General interdisciplinary view of the inactivation of $H_2S$*

Several potential mechanisms exist for the degradation of $H_2S$ in biologic samples. $H_2S$ is oxidized in mitochondria to thiosulfate (4, 34, 72), methylated in the cytosol to methanethiol and dimethylsulfate (69), and sequestered with macromolecules (56). To determine the mechanism by which endogenous $H_2S$ is degraded, it is useful to examine the molecular fate of $H_2{}^{[35]}S$ applied to tissues. Tracer delivered systemically is recovered as sulfate excreted in the urine (10). When incubated with homogenized tissue samples, or vascularly perfused through the colon, liver, or lung, >90% of the tracer is recovered as either thiosulfate or sulfate, with no evidence of methanethiol production (3, 34). Levitt and colleagues (34) found that within 1 min of delivering $H_2{}^{[35]}S$ to the lumen of the colon in rats, the tracer is recovered as thiosulfate in the portal vein, and as sulfate from blood in the heart (34). They concluded from these studies that tissues oxidize $H_2S$ to thiosulfate, which, after one pass through the liver, is oxidized further to sulfate. Therefore, the likely enzyme that inactivates $H_2S$ is a sulfide oxidase expressed in tissues.

The microbiology of sulfur-oxidizing bacteria provides some clues regarding vertebrate degradation of $H_2S$ through oxidation (14). In early studies of intestinal $H_2S$ gas, Lehmann (33) noticed that $H_2S$, although found in high concentrations in the proximal colon, was always reduced in the flatus and was never observed in blood, suggesting that a system of $H_2S$ degradation occurs. The first demonstration of biologic sulfide oxidation came from Winogradsky in 1889 (73). Animals from sulfide-rich habitats exhibit aerobic chemotropic electron transport or anaerobic phototropic electron transport through sulfide oxidation (20). Although oxidation of elemental sulfur and thiosulfate occurs in prokaryotic bacteria through the sulfur oxidase (*sox*) family of genes, especially the *soxC/D* gene (15), the major biochemical pathway of $H_2S$ oxidation in eukaryotes is by sulfide quinone reductase (SQR) (20). The crystal structure of bacterial SQR was recently solved by two independent groups (8, 42) (Fig. 6). No transmembrane domains of this protein exist, but rather, it is embedded in the matrix side of the inner membrane of the

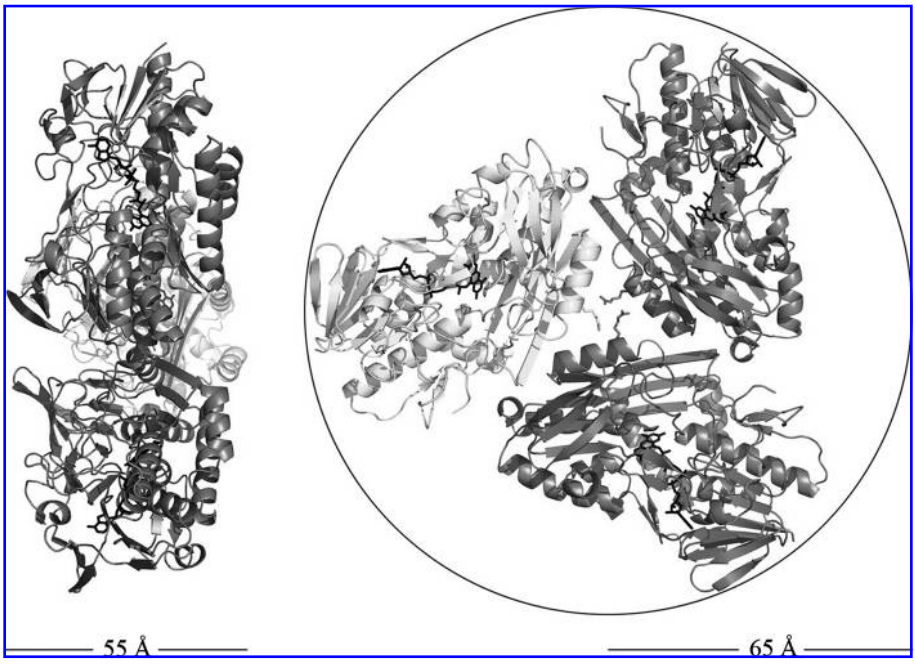


**FIG. 6. Ribbon diagram of a trimer of *Aquifex aeolicus* sulfide quinone reductase (SQR) derived from x-ray crystallography data.** Each monomer is colored in slightly different shades of gray. Flavin adenine dinucleotide (FAD) is shown in black. Reproduced with permission from Marcia and colleagues (42).

mitochondrion. The protein, as a member of the disulfide oxidoreductase flavoprotein (DiSR) superfamily, contains an FAD-binding site that positions FAD adjacent to three cysteine residues that are thought to be the catalytic site of the enzyme. A quinine-binding site is on the *si*-face of FAD, which accepts the electrons given up by $H_2S$ as they create sulfane sulfur that exists briefly as persulfides on SQR cysteine residues. In bacteria, the reaction may stop here, with the elemental sulfur contained in the persulfides being used to generate large polysulfides (8).

Most of our knowledge regarding $H_2S$ degradation in vertebrates comes from the detoxification of $H_2S$ by the colonic mucosa. Because $H_2S$ concentrations in excess of 1,000 ppm have been measured in gas samples obtained from the rat cecum (59), and $H_2S$ can readily cross membrane barriers, a detoxification pathway must exist. Whereas Levitt and colleagues (34) suggested a sulfide oxidase of unknown molecular identity, Picton and colleagues (53) suggested that thiosulfate sulfurtransferase (TST), or rhodanese, was the major enzymatic-degradation pathway. Since its discovery, rhodanese, expressed by the liver, has been known as the enzyme responsible for cyanide detoxification. Cyanide is transferred to thiosulfate to create thiocyanate and sulfite. Picton and colleagues (52) used cyanide in colonic mucosal preparations, showed that thiocyanate is the major product of $H_2S$ degradation, and used this reaction as a surrogate for measuring $H_2S$ consumption to suggest that detoxification is impaired during colitis (52). After reexamination of their data, it appears that TST is involved in $H_2S$ oxidation only when cyanide is present (72) but is likely involved in formation of thiosulfate from oxidized sulfane persulfides (21).

The vertebrate enzyme that exhibits sulfide oxidase activity appears to be SQR (21). Human SQR is encoded by the gene SQR domain-like (SQRDL), which is located on chromosome 15 (15q15; Entrez GeneID: 58472). Oxidation from $H_2S$ to sulfane persulfides on SQR occurs as described earlier for bacteria, but instead of the creation of polysulfides, other enzymes are involved to create thiosulfate. The proposed mechanism is that some of the persulfides are oxidized further to sulfite through the action of the enzyme sulfur dioxygenase, which currently does not have a molecular identity that matches its enzymatic activity. The resultant sulfite reacts with persulfides on SQR through the enzymatic action of TST to generate thiosulfate, which is excreted from the cell by an unknown mechanism. It is important to note that in native conditions, no detectable intermediates exist between $H_2S$ and thiosulfate, such that these three enzymes appear to work together so that the complete oxidation of $H_2S$ to thiosulfate occurs instantaneously (21).

Several studies demonstrated that the oxidation of $H_2S$ by SQR is sensitive to the levels of $H_2S$ (20, 21). Concentrations of $H_2S$ in excess of 300 $\mu M$ reduce the ability of SQR to oxidize $H_2S$. The biochemistry of this enzyme might underlie the apparently differential effects of slow and fast $H_2S$-releasing compounds. Quinone-based antibiotics are potent inhibitors of SQR by inhibiting the ability of the enzyme to transfer electrons to quinone (20).

Inactivation of $H_2S$ in the gastrointestinal tract. The mucosa of the gastrointestinal tract, especially the colon, is a model system of $H_2S$ degradation because of its role in detoxifying bacterially derived $H_2S$ and thus has been incorporated into the previous discussion. The inactivation of endogenously derived $H_2S$ in the gastrointestinal tract remains unknown. Recent experiments in our laboratory show that stigmatellin (3 $\mu M$) can inhibit $H_2S$ consumption (Fig. 7). These data implicate SQR in the degradation of $H_2S$ by vertebrate tissues. Because the structure of the catalytic domain of SQR was recently identified (8, 42), it is reasonable to expect that new and more-specific inhibitors of SQR will be forthcoming.

Summary of the Fifth Criterion. Based on isotope-tracing studies, it seems that the major inactivation of $H_2S$ signaling is through oxidation to thiosulfate. The mitochondrial matrix protein SQR is likely the molecular identity of sulfide oxidase responsible for catalyzing this reaction. Therefore, a specific enzymatic-degradation pathway inactivates $H_2S$, fulfilling the fifth criterion. What remains to be determined is whether pharmacologic manipulation of sulfide oxidation alters endogenous $H_2S$ signaling. The development of new SQR inhibitors as pharmacologic tools will be helpful.

## Future Directions

The pharmacologic effects of $H_2S$ generated from NaHS on intracellular metabolic and second-messenger systems, on posttranslational modification of membrane ion channels and transporters and in cell-to-cell communication, are now regarded as established and credible. The lack of solid data and evidence on the function of endogenously generated $H_2S$ gas in the living intact organism is a major impediment to providing an essential link to clinical situations. CBS-knockout mice have hyperhomocysteinemia but lack a demonstrable phenotype that suggests an endogenous role of $H_2S$ produced by CBS (68). Perhaps the absence of an $H_2S$-related phenotype

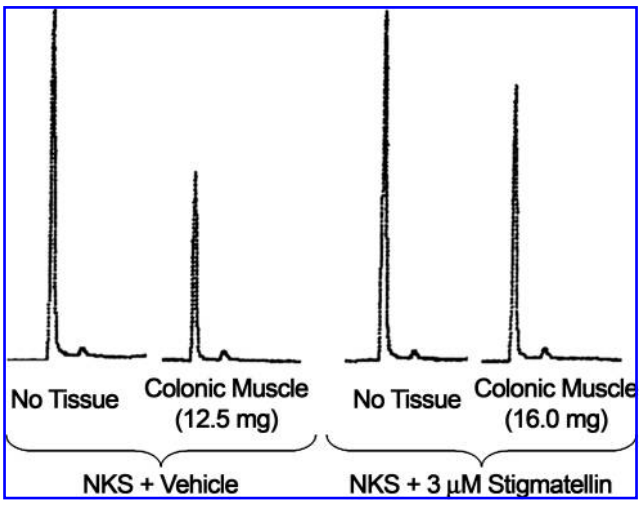


**FIG. 7. Facsimiles of representative elution peaks of $H_2S$ by using gas chromatography and sulfur fluorescence detection, demonstrating that consumption of $H_2S$ by the external muscle layers of the colon was partially inhibited by the SQR inhibitor stigmatellin.** Mouse colonic muscle tissue was incubated in 40 $\mu$l normal Kreb's solution with either 3 $\mu M$ stigmatellin or 0.1% ethanol (vehicle) in 20 ml of 3.6 ppm $H_2S$ gas in 97% $O_2$ and 3% $CO_2$. After 30 min of incubation at 37°C, the gas space was assayed for $H_2S$.

is due to the compensatory role of 3MST in the brain (23). A recent report of mice lacking CSE demonstrates phenotypic effects on blood pressure, supporting a role for endogenous $H_2S$ in the vascular system (75), but phenotypes in other systems, including the gastrointestinal tract, have not been described. Although considerable data exist in animal models on the pharmacologic effects of $H_2S$ and of sulfide-derived molecules in ischemia/reperfusion, Cardioprotection, and a variety of inflammatory conditions, the link to the clinic and the patient requires further study.

Answers to the following questions are required for $H_2S$ to join the list as one of the body's important regulators of physiologic function:

1. What triggers CBS, CSE, and 3MST to generate $H_2S$?
2. Can endogenously produced $H_2S$ be monitored in real time with probes in intact and living tissue, thereby providing spatiotemporal information regarding the concentration of $H_2S$ in the environment of the cells?
3. Do receptors for $H_2S$ demonstrate saturable, reversible binding that can be antagonized in a competitive manner?
4. Can stable $H_2S$-based molecules, rather than $H_2S$ donors, be synthesized so that classic methods to investigate ligand–receptor interactions, such as radioligand binding, be used to define $H_2S$ receptors?
5. Do the catabolic enzymes that convert $H_2S$ to inactive sulfate form an effective barrier and metabolic compartmentalization, greatly limiting local diffusion and intercellular communication?

Looking beyond $H_2S$ to the field of gaseous signaling molecules in general, ammonia and methane may in time enter the stage as gaseous signaling molecules. In addition to the critical role that renal ammonium production and excretion play in acid secretion, and as a principal nitrogen source for microorganisms and plants, it functions as a morphogen during the development of slime molds (78). Just as the active moiety of $H_2S$ appears in some instances to be $HS^-$, the ammonium ion ($NH_4^+$) has important physiologic ramifications. $NH_4^+$ transporters have been described in glial cells of the bee retina, where they appear to play an important role in energy metabolism (41). Like $HS^-$, $NH_4^+$ produces a robust intracellular acidification, and $NH_4^+$ is endogenously generated and released into the intercellular space of living tissue (9). Methane is potentially another gaseous signaling molecule. Methane slows intestinal transit in the dog and increases the contractile strength of the guinea pig ileum *in vitro* (54). No evidence is available that methane is produced by endogenous tissues, but it is produced by commensal bacteria, and bowel cleansing but not antibiotic treatment reduces exhaled methane (51).

## Summary and Conclusions

Several lines of evidence suggest that $H_2S$ is an endogenous gaseous signal molecule in the gastrointestinal tract, but work still remains. Whereas $H_2S$ is enzymatically produced, no evidence indicates that its production is regulated. Although exogenous $H_2S$ exerts several well-defined physiologic effects in the gastrointestinal tract, no study has demonstrated a receptor for $H_2S$. An enzymatic pathway exists in gastrointestinal tissues for the degradation of $H_2S$ through oxidation, but no study has demonstrated that manipulation of sulfide oxidation alters $H_2S$ signaling. Future work in the field of gasotransmitters is likely to be demanding. A critical need exists to develop novel technologies, such as $H_2S$ probes and inhibitors of the synthetic and catabolic enzymes. The road ahead for the field remains unclear, but as work in $H_2S$ biology progresses, more surprises and exciting results are expected.

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## References

1. Abe K and Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J Neurosci* 16: 1066–1071, 1996.
2. Aita N, Ishii K, Akamatsu Y, Ogasawara Y, and Tanabe S. Cloning and expression of human liver rhodanese cDNA. *Biochem Biophys Res Commun* 231: 56–60, 1997.
3. Bartholomew TC, Powell GM, Dodgson KS, and Curtis CG. Oxidation of sodium sulphide by rat liver, lungs and kidney. *Biochem Pharmacol* 29: 2431–2437, 1980.
4. Beauchamp RO Jr, Bus JS, Popp JA, Boreiko CJ, and Andjelkovich DA. A critical review of the literature on hydrogen sulfide toxicity. *Crit Rev Toxicol* 13: 25–97, 1984.
5. Bellamy TC and Garthwaite J. The receptor-like properties of nitric oxide-activated soluble guanylyl cyclase in intact cells. *Mol Cell Biochem* 230: 165–176, 2002.
6. Billaut-Laden I, Rat E, Allorge D, Crunelle-Thibaut A, Cauffiez C, Chevalier D, Lo-Guidice JM, and Broly F. Evidence for a functional genetic polymorphism of the human mercaptopyruvate sulfurtransferase (MPST), a cyanide detoxification enzyme. *Toxicol Lett* 165: 101–111, 2006.
7. Brancaleone V, Roviezzo F, Vellecco V, De GL, Bucci M, and Cirino G. Biosynthesis of $H_2S$ is impaired in non-obese diabetic (NOD) mice. *Br J Pharmacol* 155: 673–680, 2008.
8. Brito JA, Sousa FL, Stelter M, Bandeiras TM, Vonrhein C, Teixeira M, Pereira MM, and Archer M. Structural and functional insights into sulfide:quinone oxidoreductase. *Biochemistry* 48: 5613–5622, 2009.
9. Coles JA, Martiel JL, and Laskowska K. A glia-neuron alanine/ammonium shuttle is central to energy metabolism in bee retina. *J Physiol* 586: 2077–2091, 2008.
10. Curtis CG, Bartholomew TC, Rose FA, and Dodgson KS. Detoxication of sodium 35 S-sulphide in the rat. *Biochem Pharmacol* 21: 2313–2321, 1972.
11. Doeller JE, Isbell TS, Benavides G, Koenitzer J, Patel H, Patel RP, Lancaster JR Jr, Darley-Usmar VM, and Kraus DW. Polarographic measurement of hydrogen sulfide production and consumption by mammalian tissues. *Anal Biochem* 341: 40–51, 2005.
12. Dombkowski RA, Russell MJ, and Olson KR. Hydrogen sulfide as an endogenous regulator of vascular smooth muscle tone in trout. *Am J Physiol Regul Integr Comp Physiol* 286: R678–R685, 2004.
13. Fiorucci S, Antonelli E, Distrutti E, Rizzo G, Mencarelli A, Orlandi S, Zanardo R, Renga B, Di SM, Morelli A, Cirino G, and Wallace JL. Inhibition of hydrogen sulfide generation contributes to gastric injury caused by anti-inflammatory nonsteroidal drugs. *Gastroenterology* 129: 1210–1224, 2005.

14. Friedrich CG. Physiology and genetics of sulfur-oxidizing bacteria. *Adv Microb Physiol* 39: 235–289, 1998.
15. Friedrich CG, Bardischewsky F, Rother D, Quentmeier A, and Fischer J. Prokaryotic sulfur oxidation. *Curr Opin Microbiol* 8: 253–259, 2005.
16. Furne J, Saeed A, and Levitt MD. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. *Am J Physiol Regul Integr Comp Physiol* 295: R1479–R1485, 2008.
17. Gallego D, Clave P, Donovan J, Rahmati R, Grundy D, Jimenez M, and Beyak MJ. The gaseous mediator, hydrogen sulphide, inhibits in vitro motor patterns in the human, rat and mouse colon and jejunum. *Neurogastroenterol Motil* 20: 1306–1316, 2008.
18. Gayon U and Note de MU. Gayon presentee par M. Pasteur. *Comp Rend Acad Sci* 85: 1074–1076, 1877.
19. Ge Y, Konrad MA, Matherly LH, and Taub JW. Transcriptional regulation of the human cystathionine beta-synthase-1b basal promoter: synergistic transactivation by transcription factors NF-Y and Sp1/Sp3. *Biochem J* 357: 97–105, 2001.
20. Griesbeck C, Hauska G, and Schutz M. Biological sulfide oxidation: sulfide-quinone reductase (SQR), the primary reaction. In: *Recent Research Developments in Microbiology*, edited by Pandalai SG. Trivadrum, India: Research Signpost, 2000, pp. 179–203.
21. Hildebrandt TM and Grieshaber MK. Three enzymatic activities catalyze the oxidation of sulfide to thiosulfate in mammalian and invertebrate mitochondria. *FEBS J* 275: 3352–3361, 2008.
22. Hosoki R, Matsuki N, and Kimura H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem Biophys Res Commun* 237: 527–531, 1997.
23. Ishigami M, Hiraki K, Umemura K, Ogasawara Y, Ishii K, and Kimura H. A source of hydrogen sulfide and a mechanism of its release in the brain. *Antioxid Redox Signal* 11: 205–214, 2009.
24. Jacobs MB, Braverman MM, and Hochheiser S. Ultramicrodetermination of sulfides in air. *Anal Chem* 29: 1349–1351, 1957.
25. Kamoun P. Mental retardation in Down syndrome: a hydrogen sulfide hypothesis. *Med Hypotheses* 57: 389–392, 2001.
26. Kenakin T. *Pharmacologic Analysis of Drug-Receptor Interaction.* Philadelphia: Lippincott-Raven, 1997.
27. Kim YD, Seo JK, Park C-G, Kim MW, Choi S, and Jun JY. Effects of hydrogen sulfide on interstitial cells of Cajal from mouse small intestine. *Gastroenterology* 136: (T1802) A-583, 2009.
28. Kraus JP, Hasek J, Kozich V, Collard R, Venezia S, Janosikova B, Wang J, Stabler SP, Allen RH, Jakobs C, Finn CT, Chien YH, Hwu WL, Hegele RA, and Mudd SH. Cystathionine gamma-lyase: clinical, metabolic, genetic, and structural studies. *Mol Genet Metab* 97: 250–259, 2009.
29. Kraus JP, Janosik M, Kozich V, Mandell R, Shih V, Sperandeo MP, Sebastio G, de Franchis R, Andria G, Kluijtmans LA, Blom H, Boers GH, Gordon RB, Kamoun P, Tsai MY, Kruger WD, Koch HG, Ohura T, and Gaustadnes M. Cystathionine beta-synthase mutations in homocystinuria. *Hum Mutat* 13: 362–375, 1999.
30. Kraus JP, Oliveriusova J, Sokolova J, Kraus E, Vlcek C, de Franchis R, Maclean KN, Bao L, Bukovsk, Patterson D, Paces V, Ansorge W, and Kozich V. The human cystathionine beta-synthase (CBS) gene: complete sequence, alternative splicing, and polymorphisms. *Genomics* 52: 312–324, 1998.
31. Lee H, Kho HS, Chung JW, Chung SC, and Kim YK. Volatile sulfur compounds produced by *Helicobacter pylori*. *J Clin Gastroenterol* 40: 421–426, 2006.
32. Lee SW, Cheng Y, Moore PK, and Bian JS. Hydrogen sulphide regulates intracellular pH in vascular smooth muscle cells. *Biochem Biophys Res Commun* 358: 1142–1147, 2007.
33. Lehmann CG. Contents of the intestinal canal. In: *Physiological Chemistry*, translated by George E. Day. London: Harrison and Sons, 1853, pp. 128–133.
34. Levitt MD, Furne J, Springfield J, Suarez F, and DeMaster E. Detoxification of hydrogen sulfide and methanethiol in the cecal mucosa. *J Clin Invest* 104: 1107–1114, 1999.
35. Li L and Moore PK. Putative biological roles of hydrogen sulfide in health and disease: a breath of not so fresh air? *Trends Pharmacol Sci* 29: 84–90, 2008.
36. Linden DR, Sha L, Mazzone A, Stoltz GJ, Bernard CE, Furne JK, Levitt MD, Farrugia G, and Szurszewski JH. Production of the gaseous signal molecule hydrogen sulfide in mouse tissues. *J Neurochem* 106: 1577–1585, 2008.
37. Lu Y, O'Dowd BF, Orrego H, and Israel Y. Cloning and nucleotide sequence of human liver cDNA encoding for cystathionine gamma-lyase. *Biochem Biophys Res Commun* 189: 749–758, 1992.
38. Maclean KN, Janosik M, Kraus E, Kozich V, Allen RH, Raab BK, and Kraus JP. Cystathionine beta-synthase is coordinately regulated with proliferation through a redox-sensitive mechanism in cultured human cells and *Saccharomyces cerevisiae*. *J Cell Physiol* 192: 81–92, 2002.
39. Macpherson LJ, Dubin AE, Evans MJ, Marr F, Schultz PG, Cravatt BF, and Patapoutian A. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature* 445: 541–545, 2007.
40. Maeda Y, Aoki Y, Sekiguchi F, Matsunami M, Takahashi T, Nishikawa H, and Kawabata A. Hyperalgesia induced by spinal and peripheral hydrogen sulfide: evidence for involvement of Cav3.2 T-type calcium channels. *Pain* 142: 127–132, 2009.
41. Marcaggi P and Coles JA. A little bit of ammonium may be good for your brain. *Physiol News* 73: 15–17, 2008.
42. Marcia M, Ermler U, Peng G, and Michel H. The structure of *Aquifex aeolicus* sulfide:quinone oxidoreductase: a basis to understand sulfide detoxification and respiration. *Proc Natl Acad Sci U S A* 106: 9625–9630, 2009.
43. Matsunami M, Tarui T, Mitani K, Nagasawa K, Fukushima O, Okubo K, Yoshida S, Takemura M, and Kawabata A. Luminal hydrogen sulfide plays a pronociceptive role in mouse colon. *Gut* 58: 751–761, 2009.
44. Meier M, Janosik M, Kery V, Kraus JP, and Burkhard P. Structure of human cystathionine beta-synthase: a unique pyridoxal 5′-phosphate-dependent heme protein. *EMBO J* 20: 3910–3916, 2001.
45. Mellor JW. *A Comprehensive Treatise on Inorganic and Theoretical Chemistry.* London: Logmans, Green and Co, 1930.
46. Mustafa AK, Gadalla MM, and Snyder SH. Signaling by gasotransmitters. *Sci Signal* 2: re2(68), 2009.
47. Nelson ME, Kuryatov A, Choi CH, Zhou Y, and Lindstrom J. Alternate stoichiometries of alpha4beta2 nicotinic acetylcholine receptors. *Mol Pharmacol* 63: 332–341, 2003.
48. Nicholls P, Petersen LC, Miller M, and Hansen FB. Ligand-induced spectral changes in cytochrome c oxidase and their possible significance. *Biochim Biophys Acta* 449: 188–196, 1976.
49. Olson KR, Healy MJ, Qin Z, Skovgaard N, Vulesevic B, Duff DW, Whitfield NL, Yang G, Wang R, and Perry SF. Hy-

drogen sulfide as an oxygen sensor in trout gill chemoreceptors. *Am J Physiol Regul Integr Comp Physiol* 295: R669–R680, 2008.
50. Pallini R, Guazzi GC, Cannella C, and Cacace MG. Cloning and sequence analysis of the human liver rhodanese: comparison with the bovine and chicken enzymes. *Biochem Biophys Res Commun* 180: 887–893, 1991.
51. Peled Y, Weinberg D, Hallak A, and Gilat T. Factors affecting methane production in humans: gastrointestinal diseases and alterations of colonic flora. *Dig Dis Sci* 32: 267–271, 1987.
52. Picton R, Eggo MC, Langman MJ, and Singh S. Impaired detoxication of hydrogen sulfide in ulcerative colitis? *Dig Dis Sci* 52: 373–378, 2007.
53. Picton R, Eggo MC, Merrill GA, Langman MJ, and Singh S. Mucosal protection against sulphide: importance of the enzyme rhodanese. *Gut* 50: 201–205, 2002.
54. Pimentel M, Lin HC, Enayati P, van den BB, Lee HR, Chen JH, Park S, Kong Y, and Conklin J. Methane, a gas produced by enteric bacteria, slows intestinal transit and augments small intestinal contractile activity. *Am J Physiol Gastrointest Liver Physiol* 290: G1089–G1095, 2006.
55. Schicho R, Krueger D, Zeller F, Von Weyhern CW, Frieling T, Kimura H, Ishii I, De GR, Campi B, and Schemann M. Hydrogen sulfide is a novel prosecretory neuromodulator in the guinea-pig and human colon. *Gastroenterology* 131: 1542–1552, 2006.
56. Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, Ishii K, and Kimura H. 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid Redox Signal* 11: 703–714, 2008.
57. Stipanuk MH. Sulfur amino acid metabolism: pathways for production and removal of homocysteine and cysteine. *Annu Rev Nutr* 24: 539–577, 2004.
58. Stipanuk MH and Beck PW. Characterization of the enzymic capacity for cysteine desulphhydration in liver and kidney of the rat. *Biochem J* 206: 267–277, 1982.
59. Suarez F, Furne J, Springfield J, and Levitt M. Production and elimination of sulfur-containing gases in the rat colon. *Am J Physiol* 274: G727–G733, 1998.
60. Sullivan JM, Traynelis SF, Chen HS, Escobar W, Heinemann SF, and Lipton SA. Identification of two cysteine residues that are required for redox modulation of the NMDA subtype of glutamate receptor. *Neuron* 13: 929–936, 1994.
61. Sun Q, Collins R, Huang S, Holmberg-Schiavone L, Anand GS, Tan CH, van-den-Berg S, Deng LW, Moore PK, Karlberg T, and Sivaraman J. Structural basis for the inhibition mechanism of human cystathionine gamma-lyase, an enzyme responsible for the production of H(2)S. *J Biol Chem* 284: 3076–3085, 2009.
62. Szabo C. Hydrogen sulphide and its therapeutic potential. *Nat Rev Drug Discov* 6: 917–935, 2007.
63. Tangerman A. Measurement and biological significance of the volatile sulfur compounds hydrogen sulfide, methanethiol and dimethyl sulfide in various biological matrices. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009. [Epub ahead of print].
64. Teague B, Asiedu S, and Moore PK. The smooth muscle relaxant effect of hydrogen sulphide in vitro: evidence for a physiological role to control intestinal contractility. *Br J Pharmacol* 137: 139–145, 2002.
65. Ullian ME, Gelasco AK, Fitzgibbon WR, Beck CN, and Morinelli TA. *N*-acetylcysteine decreases angiotensin II receptor binding in vascular smooth muscle cells. *J Am Soc Nephrol* 16: 2346–2353, 2005.
66. Wang J and Hegele RA. Genomic basis of cystathioninuria (MIM 219500) revealed by multiple mutations in cystathionine gamma-lyase (CTH). *Hum Genet* 112: 404–408, 2003.
67. Wang R. Two's company, three's a crowd: Can $H_2S$ be the third endogenous gaseous transmitter? *FASEB J* 16: 1792–1798, 2002.
68. Watanabe M, Osada J, Aratani Y, Kluckman K, Reddick R, Malinow MR, and Maeda N. Mice deficient in cystathionine beta-synthase: animal models for mild and severe homocyst(e)inemia. *Proc Natl Acad Sci U S A* 92: 1585–1589, 1995.
69. Weisiger RA, Pinkus LM, and Jakoby WB. Thiol S-methyltransferase: suggested role in detoxication of intestinal hydrogen sulfide. *Biochem Pharmacol* 29: 2885–2887, 1980.
70. Werman R. Criteria for identification of a central nervous system transmitter. *Comp Biochem Physiol* 18: 745–766, 1966.
71. Whitfield NL, Kreimier EL, Verdial FC, Skovgaard N, and 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* 294: R1930–R1937, 2008.
72. Wilson K, Mudra M, Furne J, and Levitt M. Differentiation of the roles of sulfide oxidase and rhodanese in the detoxification of sulfide by the colonic mucosa. *Dig Dis Sci* 53: 277–283, 2008.
73. Winogradsky S. Recherches physiologiques sur les sulfobacteries. *Ann Inst Pasteur (Paris)* 3: 49–60, 1889.
74. Yamada H, Otsuka M, Fujimoto K, Kawashima K, and Yoshida M. Determination of acetylcholine concentration in cerebrospinal fluid of patients with neurologic diseases. *Acta Neurol Scand* 93: 76–78, 1996.
75. Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, and Wang R. $H_2S$ as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science* 322: 587–590, 2008.
76. Yang W, Yang G, Jia X, Wu L, and Wang R. Activation of KATP channels by $H_2S$ in rat insulin-secreting cells and the underlying mechanisms. *J Physiol* 569: 519–531, 2005.
77. Yong QC, Lee SW, Foo CS, Neo KL, Chen X, and Bian JS. Endogenous hydrogen sulphide mediates the cardioprotection induced by ischemic postconditioning. *Am J Physiol Heart Circ Physiol* 295: H1330–H1340, 2008.
78. Yoshino R, Morio T, Yamada Y, Kuwayama H, Sameshima M, Tanaka Y, Sesaki H, and Iijima M. Regulation of ammonia homeostasis by the ammonium transporter amtA in *Dictyostelium discoideum*. *Eukaryot Cell* 6: 2419–2428, 2007.
79. Zhao W, Ndisang JF, and Wang R. Modulation of endogenous production of $H_2S$ in rat tissues. *Can J Physiol Pharmacol* 81: 848–853, 2003.
80. Zhao W, Zhang J, Lu Y, and Wang R. The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. *EMBO J* 20: 6008–6016, 2001.

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**Abbreviations Used**

3MST = 3-mercaptopyruvate sulfurtransferase
cAMP = cyclic adenosine monophosphate
CBS = cystathionine-$\beta$-synthase
CNS = central nervous system
CO = carbon monoxide
CSE = cystathione-$\gamma$-lyase
EDRF = epithelium-derived relaxing factor
FAD = flavin adenine dinucleotide
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
$H_2S$ = hydrogen sulfide
ICCs = interstitial cells of Cajal
KATP = ATP-sensitive potassium channel
NMDA = *N*-methyl-D-aspartic acid
NO = nitric oxide
ODQ = 1H[1,2,4] oxadiazolo-[4,3-$\gamma$] quinoxalin-1-one
PLP = pyroxidyl-5-phosphate
sGC = soluble guanylate cyclase
SQR = sulfide quinone reductase
TRP = transient receptor potential

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2. Chris Peers , Claudia C. Bauer , John P. Boyle , Jason L. Scragg , Mark L. Dallas . 2012. Modulation of Ion Channels by Hydrogen Sulfide. *Antioxidants & Redox Signaling* **17**:1, 95-105. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
3. Kenneth R. Olson . 2012. A Practical Look at the Chemistry and Biology of Hydrogen Sulfide. *Antioxidants & Redox Signaling* **17**:1, 32-44. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. Michael S. Kasparek, David R. Linden, Gianrico Farrugia, Michael G. Sarr. 2012. Hydrogen Sulfide Modulates Contractile Function in Rat Jejunum. *Journal of Surgical Research* **175**:2, 234-242. [CrossRef]
5. Kenneth R. Olson. 2012. Mitochondrial adaptations to utilize hydrogen sulfide for energy and signaling. *Journal of Comparative Physiology B* . [CrossRef]
6. D. R. Linden, J. Furne, G. J. Stoltz, M. S. Abdel-Rehim, M. D. Levitt, J. H. Szurszewski. 2011. Sulfide quinone reductase contributes to hydrogen sulfide metabolism in murine peripheral tissues but not in the central nervous system. *British Journal of Pharmacology* no-no. [CrossRef]
7. Ling Li, Peter Rose, Philip K. Moore. 2011. Hydrogen Sulfide and Cell Signaling. *Annual Review of Pharmacology and Toxicology* **51**:1, 169-187. [CrossRef]
8. F. Ise, H. Takasuka, S. Hayashi, K. Takahashi, M. Koyama, E. Aihara, K. Takeuchi. 2011. Stimulation of duodenal HCO3- secretion by hydrogen sulphide in rats: relation to prostaglandins, nitric oxide and sensory neurones. *Acta Physiologica* **201**:1, 117-126. [CrossRef]
9. Munenori Nagao, David R. Linden, Judith A. Duenes, Michael G. Sarr. 2011. Mechanisms of Action of the Gasotransmitter Hydrogen Sulfide in Modulating Contractile Activity of Longitudinal Muscle of Rat Ileum. *Journal of Gastrointestinal Surgery* **15**:1, 12-22. [CrossRef]
10. M. Jimenez. 2010. Hydrogen sulfide as a signaling molecule in the enteric nervous system. *Neurogastroenterology & Motility* **22**:11, 1149-1153. [CrossRef]
11. D. Krueger, M. Foerster, K. Mueller, F. Zeller, J. Slotta-huspenina, J. Donovan, D. Grundy, M. Schemann. 2010. Signaling mechanisms involved in the intestinal pro-secretory actions of hydrogen sulfide. *Neurogastroenterology & Motility* **22**:11, 1224-e320. [CrossRef]
12. H. I. Akbarali, E. G. Hawkins, G. R. Ross, M. Kang. 2010. Ion channel remodeling in gastrointestinal inflammation. *Neurogastroenterology & Motility* **22**:10, 1045-1055. [CrossRef]
13. Rui Wang . 2010. Hydrogen Sulfide: The Third Gasotransmitter in Biology and Medicine. *Antioxidants & Redox Signaling* **12**:9, 1061-1064. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]