

EXPERT
REVIEWS

Hydrogen sulfide as a potential biomarker of asthma

Expert Rev. Respir. Med. 8(1), 5–13 (2014)

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Hydrogen sulfide (H₂S), a gas characterized by the odor of rotten eggs, is produced by many cells in the airways and lungs, and may regulate physiologic and pathophysiologic processes. It plays a role in cellular signaling, and represents the third gasotransmitter after nitric oxide and carbon monoxide. Endogenous and exogenous H₂S have anti-inflammatory and anti-proliferative effects, with inhibitory effects in models of lung inflammation and fibrosis. Under certain conditions, H₂S may also be proinflammatory. It is generally a vasodilator and relaxant of airway and vascular smooth muscle cells. It acts as a reducing agent, being able to scavenge superoxide and peroxynitrite. H₂S is detectable in serum and in sputum supernatants with raised levels observed in asthmatics. The sputum levels correlated inversely with lung function. H₂S may play a role in the pathogenesis of asthma.

KEYWORDS: asthma • hydrogen sulfide • lung inflammation • nitric oxide

Asthma is characterized by a chronic airway inflammatory process involving a range of inflammatory cells including eosinophils and mast cells, and by bronchial hyperresponsiveness and bronchodilator responses to β -adrenergic agonists. This inflammatory profile can vary from patient to patient such as eosinophilic- or neutrophil-predominant inflammation, and the clinical significance of these inflammatory subtypes are still being determined [1,2]. The presence of eosinophilic inflammation as measured in sputum samples may be indicative of good therapeutic response to corticosteroid therapy, while that of a neutrophilic response reflecting more severe asthma and possibly, corticosteroid insensitivity [3–5]. Asthma can also be categorized according to its severity, and there is increasing interest in severe asthma patients who are refractory to current asthma medications [6,7]. It is now becoming increasingly recognized that patients with asthma may fall into distinct categories or phenotypes according to their clinical presentation and inflammatory characteristics [8,9]. With this recognition, it is becoming important to determine whether there are biomarkers that would be able to distinguish these different phenotypes of asthma. While assessment of sputum eosinophils has been useful in defining eosinophilic asthma, the definition of

non-eosinophilic neutrophilic asthma remains more problematic. This definition entirely relies on detecting an excess of neutrophils in the sputum, and what constitutes a ‘normal’ number of neutrophils in induced sputum samples remains unclear. Therefore, a more convenient biomarker for ‘neutrophilic’ asthma is required. While serum periostin levels, a product of Th2 cells, may represent a biomarker for eosinophilic asthma [10], a more convenient parallel marker for neutrophilic asthma does not exist.

In the search for biomarkers that would be specific for asthma phenotypes, the value of using levels of the gas nitric oxide (NO) in exhaled breath has been closely scrutinized. NO can now be measured from a breath exhalation and is now used as a more convenient way of detecting eosinophilic airway inflammation, assessing corticosteroid responsiveness and monitoring of airway inflammation to determine the need for corticosteroid treatment [11].

In this report, attention will be focused on another gas hydrogen sulfide (H₂S) that may well define a particular phenotype of asthma. H₂S is remembered by many from school chemistry classes as the gas that smells of rotten eggs when produced by the interaction of sulfides with various acids. It is also known as a toxic gas that can be encountered in

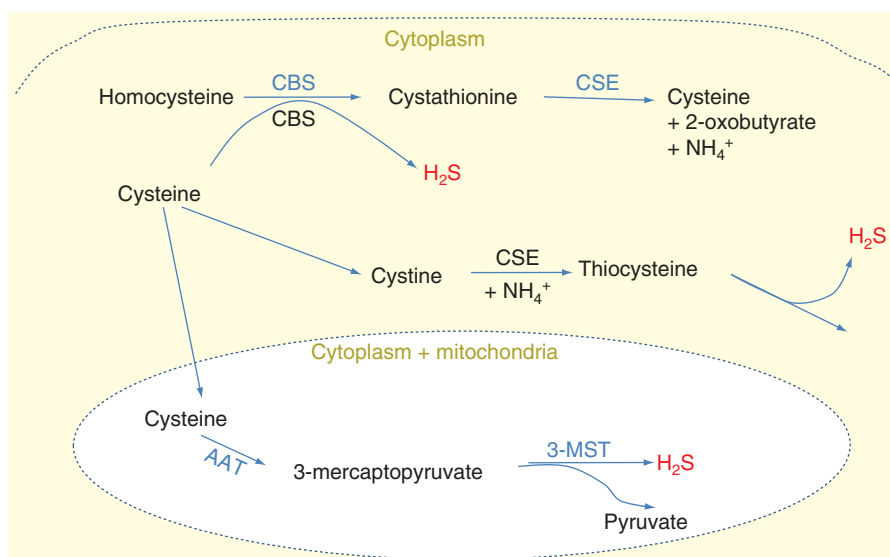


Figure 1. Intracellular synthesis of hydrogen sulfide. H₂S is synthesized mainly by three enzymes: CBS, CSE and MST. Most of the H₂S synthesis is from CSE and CBS via the metabolism of cysteine, homocysteine and cystathionine. Cysteine is also converted by AAT to 3-mercaptopyruvate, which then produces H₂S by the action of 3-MST.

AAT: Aspartate aminotransferase; CBS: Cystathionine β-synthase; CSE: Cystathionine γ-lyase; H₂S: Hydrogen sulfide; MST: Mercaptopyruvate sulfurtransferase.

sewage plants and during manure treatment procedures, and in natural gas fields with depressing effects on conscious level and respiration, while lower concentrations may induce rhinitis, pulmonary edema and acute respiratory failure. Less well known is the fact that this freely diffusible gas is produced endogenously by a variety of cells and work in the past decade has established H₂S as a gasotransmitter with physiological and pathophysiological roles in various diseases [12]. This signaling role for H₂S has led to the qualification that H₂S is the third gasotransmitter, after NO and CO [13]. Because asthma is an inflammatory disorder associated with remodeling changes, and H₂S does have properties that interfere with these processes, it does make sense to examine the role of H₂S as a biomarker in asthma. In addition, there is the developing notion that H₂S could also be used for therapeutic purposes [14,15].

H₂S synthesis

The bulk of endogenous H₂S synthesis is catalyzed from the pyridoxal-5'-phosphate-dependent enzymes, cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS), and from 3-mercaptopyruvate sulfurtransferase (3-MST) (FIGURE 1). While CBS and CSE are present in the cytosol, 3-MST is present in both cytosol and in mitochondria. CBS is found primarily in nervous tissue, whereas CSE is expressed in vascular and inflammatory cells. 3-MST in vascular endothelial cells and brain has been proposed to generate H₂S via enzymatic desulfuration of β-mercaptopyruvate derived from cysteine transamination [16,17]. CBS and CSE can generate H₂S by using many substrates. In human tissues, most of H₂S synthesis is from

CSE and CBS via the metabolism of cysteine, homocysteine and cystathionine. CBS can use cysteine and homocysteine, forming cystathionine. Under physiological conditions, this will account for most of the total H₂S generated from CBS. CSE is responsible for the hydrolytic degradation of L-cystathionine to form H₂S [18]. CSE also catalyzes β-disulfide elimination on cysteine, leading to the generation of H₂S and a disulfide Cys-S-S-Cys. 3-MST acts in combination with cysteine aminotransferase (CAT) to produce H₂S from cysteine. However, 3-MST is active usually at high pH levels but the endogenous reducing agents, thioredoxin and dihydrolipoic acid have been shown to release H₂S [19]. Its role in cells remains unclear.

There is already some information regarding the expression of H₂S-producing enzymes in lung tissues. CSE and MST proteins are widely expressed in bovine pulmonary arterial smooth muscle cells, while CBS is found only

in bovine pulmonary arterial endothelial cells as assessed by immunochemical methods [20]. Human airway smooth muscle cells and human lung primary fibroblast cells express both CSE and CBS [21,22]. In mouse lung tissues, both CSE and CBS were found in pulmonary blood vessels, smooth muscle cells and endothelial cells as well as airway smooth muscle cells [23]. Mouse peritoneal macrophages express CSE but not CBS and produce H₂S, an effect enhanced by lipopolysaccharide [24].

Anti-inflammatory effects of H₂S

H₂S may play a role with anti-inflammatory effects in promoting the resolution of inflammation. Thus, H₂S reduces adherence of leucocytes to the vascular endothelium and the extravasation of leucocytes [25]. H₂S donors reduce the infiltration of neutrophils and lymphocytes partly by inhibition of endothelial and leucocyte adhesion molecule expression [25,26]. H₂S also inhibits COX-2 expression with a reduction in PGE₂ [27]. It inhibits phosphodiesterase activity and may contribute to its anti-inflammatory actions via elevation of cyclic AMP and/or cyclic GMP [28]. H₂S can also scavenge oxidants and peroxynitrite [29,30]. It inhibits the transcription factor NF-κB and reduces proinflammatory cytokine, chemokine and enzyme (such as iNOS) expression [26,31,32]. There is also evidence for upregulation of anti-oxidant enzymes such as superoxide dismutase, glutathione peroxidase, dismutase and thioredoxin [33–35]. In terms of resolution of inflammation, H₂S can induce neutrophil apoptosis [36], and can shift macrophage function to a pro-resolution phenotype [37], with enhancement of bacterial phagocytosis and suppression of endotoxin-induced production of TNF-α.

In the ovalbumin-sensitized and challenge model, decreased levels of H₂S in pulmonary tissue in ovalbumin-treated rats were observed. Administration of sodium hydrogen sulfide (NaHS) as a source of exogenous H₂S caused a decrease in airway inflammation and airway remodeling features [38]. This anti-inflammatory effect of NaHS was reproduced in the ovalbumin allergen challenge mouse model with inhibition of eosinophil and neutrophilic lung inflammation [39]. H₂S also reduced tobacco smoke-induced lung inflammation in mice [40,41]. Another protective role for H₂S is indicated by the prevention provided by NaHS on particulate air pollution-induced endothelial disruption and lung vascular leakage, through scavenging reactive oxygen species [42]. These observations indicate a potential anti-inflammatory effect of exogenous H₂S in asthma and chronic obstructive pulmonary disease (COPD).

H₂S as proinflammatory through neurogenic inflammation

Paradoxically, H₂S has also been shown to have proinflammatory effects in animal models of LPS-induced endotoxemia and sepsis models [43,44]. Part of this effect may be due to the rapid increase in tissue H₂S concentrations. In this model, dl-propargylglycine (PPG) which irreversibly inhibits CSE attenuated neutrophil infiltration with recovery in a septic shock model, while addition of sodium sulfide aggravated it [45]. It has been suggested that the rapid release of H₂S such as provided by adding NaHS may contribute to a proinflammatory effect of H₂S, while a more slow 'physiological' release of H₂S may be less harmful or even be anti-inflammatory [15].

Part of the proinflammatory mechanism of H₂S may be through the stimulation of sensory neuropeptide release such as substance P (SP) and calcitonin gene-related peptide (CGRP) [46]. In a model of sepsis, H₂S induced systemic inflammation and multiple organ damage via transient receptor vanilloid-1 (TRPV1)-mediated neurogenic inflammation. NaHS caused atropine-resistant contraction of isolated airways which was prevented by capsaicin desensitization and which was mediated by TRPV1 receptor stimulation on sensory nerve endings [47]. NaHS caused lung inflammation in mice which was mediated through TRPV1, as evidenced by blockade by capsaizepine that caused SP release and neurogenic inflammation [48].

H₂S as a reducing agent

H₂S has reducing properties by allowing reduction of disulfide bonds and scavenging of various oxidants such as superoxide and peroxynitrite [49]. H₂S does not react readily with hydrogen peroxide [18] but it reduced peroxynitrite-mediated toxicity and tyrosine nitration [30,50]. In a hypoxic pulmonary hypertension model, H₂S reduced oxidized glutathione levels and increased total anti-oxidant capacity in lung tissues, associated with a reduction in pulmonary artery pressures [51]. H₂S decreased cigarette smoke-induced oxidative stress, inflammation and remodeling and emphysema in mice [41]. In an ovalbumin challenge mouse model, NaHS inhibited eosinophil and neutrophilic lung inflammation and prevented damage by increasing

endogenous anti-oxidant with inhibition of lipid peroxidation. It also increased superoxide dismutase, glutathione peroxidase and glutathione reductase activity, together with a reduction in iNOS [39]. This indicates that H₂S may be useful as an anti-oxidant in asthma which is a condition where there is an imbalance of oxidant–anti-oxidant influences that is at the basis of the increased oxidative stress [52]. The transcription factor, nuclear factor E2-related factor 2 (Nrf2), is important in activating anti-oxidant genes conferring protection against oxidative stress. Nrf2-anti-oxidant response elements binding was found to be reduced in airway smooth muscle cells from patients with severe asthma [53]. One of the anti-oxidant mechanisms for H₂S may lie in its ability to stabilize Nrf2 protein and induce Nrf2-target genes via an anti-oxidant/electrophile response element that inhibits the S-sulphydration of C226/C613 residues of its inhibitor, Kelch-like ECH-associated protein-1 (Keap-1) [54,55]. This is one example of modification of enzyme activity through sulphydration, through which many actions of H₂S may be mediated. Thus, H₂S has been shown to induce sulphydration of enzymes including glyceraldehyde-3-phosphate dehydrogenase, β -tubulin and actin, leading to an increase in their biologic activities [56].

Effects on cell proliferation & fibrosis

Endogenous H₂S through activation of CSE inhibits proliferation and growth of smooth muscle cells [57]. Exogenous H₂S also inhibited proliferation while at the same time inducing apoptosis of vascular smooth muscle cells [57,58]. Of more relevance to the airways, both endogenous H₂S, and exogenous H₂S delivered from a fast donor, NaHS, and a slow donor, GY44137, inhibited human airway smooth muscle cell proliferation and IL-8 release induced by fetal calf serum [22]. In these cells, the enzyme, CBS, and not CSE was mainly involved in the endogenous production of H₂S. The mechanism underlying the inhibitory effects of H₂S could be through the inhibition of phosphorylation of the MAPKs, ERK-1/2 and p38.

H₂S also has effects on fibrotic processes. It attenuated epithelial–mesenchymal transition of human alveolar epithelial cells induced by TGF- β ₁, through inhibition of Smad 2/3 phosphorylation. H₂S also suppressed human lung fibroblast proliferation induced by fetal bovine serum and PDGF-BB, and TGF- β ₂-induced myofibroblast transdifferentiation [59].

Effects on airway & vascular contractile responses

H₂S, liberated from NaHS as a donor, inhibited mouse bronchial airway smooth muscle preparations pre-contracted with carbachol very strongly, but with only a small effect on guinea-pig bronchial rings. This effect was not through the activation of ATP-sensitive K⁺ channels, of soluble guanylate cyclase, of cyclooxygenase-1 or -2, or of tachykinin receptors [60]. H₂S relaxes airway smooth muscle cells through an effect on ATP-sensitive K⁺ channels, followed by a decrease in intracellular calcium. On the other hand, H₂S has vasoconstrictor effects on vascular smooth muscle in both the systemic and pulmonary circulations [61,62].

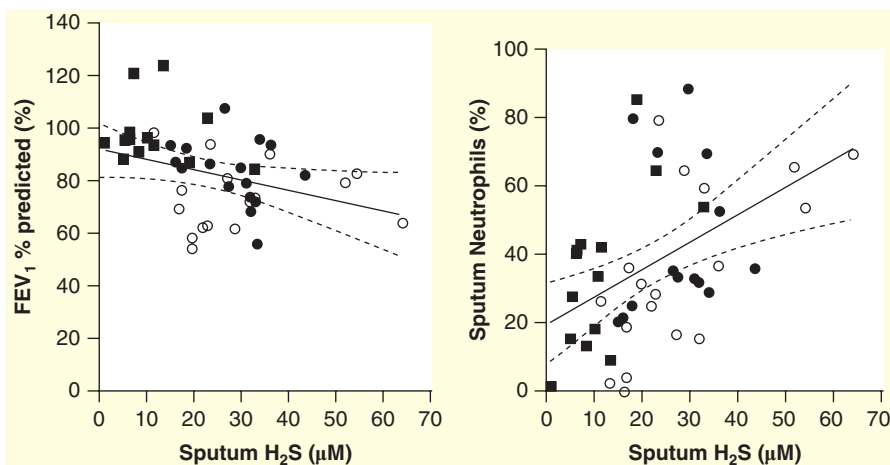


Figure 2. H₂S levels in asthma. Upper panel shows inverse relationship between sputum levels of H₂S and FEV₁ (% of predicted values) and lower panel shows a positive relationship between sputum levels of H₂S and neutrophils in sputum as percent of total cells in patients with severe asthma (open circles), non-severe asthma (solid circles) and non-asthmatic subjects (solid squares). The line of identity is shown with 95% confidence intervals.
FEV₁: Forced expiratory volume in one second; H₂S: Hydrogen sulfide.
Reproduced by permission from [71].

H₂S in hypoxic pulmonary vasoconstriction

H₂S has been implicated in hypoxic pulmonary vasoconstriction which may occur during acute episodes of asthma associated with hypoxemia. Hypoxia suppresses normal mitochondrial H₂S metabolism in an oxygen-dependent manner [63], while also promoting H₂S accumulation via cystathionine γ -lyase [64]. H₂S may minimize hypoxic vasoconstriction via inhibition by H₂S of mitochondrial oxidative phosphorylation [65], but this effect cannot be considered as a physiological role for H₂S [66]. In vascular smooth muscle cells, CSE is localized mainly in cytosol, not mitochondria. On the other hand, under conditions of a sustained increase in intracellular Ca²⁺ secondary to calcium ionophore A23187, thapsigargin or tunicamycin, CSE translocates from cytosol to mitochondria where CSE metabolizes cysteine to produce H₂S which leads to an increased ATP production in mitochondria, under hypoxic conditions [67]. Thus, H₂S production in the mitochondria provides a mechanism of resistance to the effects of hypoxia by increasing ATP synthesis.

Measurement of H₂S in asthma

To determine whether H₂S can be a potential biomarker of physiological or pathophysiological function, its measurement in body fluids is pivotal. H₂S has been measured in the circulation and was initially estimated to be approximately 10–100 μmol/l in normal volunteers [68,69]. Serum H₂S measured using a sulfide-ion selective electrode with a sensitivity of 1 μM was found to be 152 μM, a level higher than that reported in previous studies [70,71]. One of the reasons for this discrepancy might be the addition of sulfide anti-oxidant buffer which prevents the oxidation of sulfur compounds and avoids volatilization of H₂S. However, there has been criticism that the levels of H₂S in serum as

detected in μM levels may be spuriously high. Use of a polarographic sensor to measure H₂S gas found relatively low levels in blood of the order of <100 μM of total sulfide [72]. Levels of free H₂S of >1 μM would be detectable by the human nose as the estimated odor threshold for NaHS dissolved in phosphate buffer solution was 1 μM [73]. In addition, serum does not smell of H₂S and the levels detected by the sulfide-ion selective method is likely to represent the total sulfides derived by hydroxyl replacement on cysteine in the blood protein pool [72]. However, the detection of sulfides in its various forms is needed to determine the bioavailable concentrations of the various species of sulfides, particularly of H₂S, as has been recently determined [74]. Blood levels of H₂S measured by the selective ion electrode method therefore represent at best the levels of hydroxyl replacement on cysteine in the blood protein pool.

There are a few studies that have measured H₂S levels in asthma (Box 1). Levels of serum H₂S measured by the selective ion electrode method were found to be slightly reduced in patients with stable asthma; in those with an acute exacerbation of asthma, there was a further reduction in the levels of H₂S [75]. In children with asthma, serum H₂S was reduced compared with non-asthmatic children and was correlated with lung function indices [76]. These studies suggested that the levels of endogenous H₂S were reduced in asthma. However, in a recent study, serum H₂S levels were found to be elevated in patients with asthma, with no differentiation between mild and severe patients [77]. In addition, levels in induced sputum were also measured concomitantly and were found to be elevated with H₂S levels from severe and non-severe asthmatic patients (28 and 27 μM, respectively) being significantly higher than those in healthy subjects (11 μM). Serum H₂S levels were 10-times higher than in sputum and they were also higher in severe and non-severe asthmatic subjects compared with healthy subjects. These levels did not reflect salivary contamination where levels were lower and not different between the groups. There was no significant difference in sputum and serum H₂S levels between asthmatic subjects with and without inhaled corticosteroid treatment. Sputum H₂S levels were negatively correlated with FEV₁ %predicted and with reversibility to salbutamol (Figure 2). There was a positive correlation between sputum H₂S levels and % neutrophils in sputum, and an inverse correlation between sputum H₂S levels and % macrophages. These measurements need therefore to be interpreted with caution and to be confirmed with the analytical method used by Whitfield et al. [72].

Multiple linear regression analysis revealed that sputum H₂S levels were associated with increased sputum (%) neutrophil,

increased BMI and decreased %predicted FEV₁. It is therefore interesting that sputum H₂S levels could reflect a neutrophilic, obstructive type of asthma and reduced b₂-agonist bronchodilator responsiveness.

Sputum H₂S appears to be a better biomarker of asthma than serum H₂S. Since serum H₂S levels may be influenced by non-respiratory diseases such as heart, liver, renal and pancreatic disease [22], serum levels may not be specific to asthma. Increased levels of serum H₂S in asthma may represent the systemic component of the disease. Whether serum H₂S levels could represent a systemic component or is representative of 'spillover' from the lungs is not known. Sputum H₂S levels may also be influenced by many factors. The effect of asthma medications particularly β -adrenergic agonists and corticosteroids might have on sputum H₂S is not known, although corticosteroids can inhibit H₂S production in vitro partly through the inhibition of CSE [24,78]. Bacteria in the upper and lower airways may contribute to H₂S production, particularly that the lower airways contain bacteria and this bacteriome is altered in asthma [79,80]. In addition, antibiotic therapy may have an effect on sputum H₂S levels. H₂S produced by bacteria may sustain bacterial growth and suppress their sensitivity to antibiotics [81]. Thus, sputum H₂S could partly reflect the presence of bacterial infections in the airways and lungs.

H₂S levels in COPD

COPD is another condition of the airways and lungs characterized by progressive airflow limitation associated with an abnormal inflammatory response of the airways and lungs with neutrophils and macrophages and with destruction of alveoli and airway fibrosis [82]. COPD is usually considered as a separate disease from asthma, with a different airway inflammatory profile in the lungs and also accompanied by emphysema which is not usually present in asthma. Previous studies have reported levels of H₂S in serum in COPD and in chest infections [70,71]. Serum H₂S levels in stable COPD patients were found to be higher than those in healthy non-smokers but these levels were lower with acute exacerbations of COPD [70]. The effect of theophylline on serum H₂S levels in COPD patients has also been reported. Although theophylline reduced sputum neutrophils, it had no effect on serum H₂S and NO_x levels, and serum and sputum CXCL8 levels. There was a positive correlation between the FEV₁ %predicted and the level of serum H₂S [83]. These preliminary studies suggest that H₂S levels and H₂S metabolism may be altered in COPD.

Measurement of H₂S in exhaled breath

Levels of H₂S in exhaled air may provide a more sensitive biomarker of airways disease such as asthma, but more sensitive techniques such as gas chromatography using a two-stage thermal desorption capillary gas chromatography with flame ionization and flame photometric detection, or mass spectrometry have been used [84–86]. Using mass spectrometry, exhaled H₂S was found to be 47.8 ppb in healthy subjects, and increased to 55.2 ppb in patients with chronic pancreatitis [84]. In another

Box 1. Measurement of hydrogen sulfide in asthma and chronic obstructive pulmonary disease.

- Serum H₂S measured by selective ion electrode slightly reduced in stable asthma, with further reductions in those in acute exacerbation [74].
- Serum H₂S reduced in asthmatic children and levels correlated with lung function [75].
- Serum levels elevated in patients with asthma, without any differentiation by severity of asthma [76]. Levels of H₂S in sputum were also elevated [76].
- Serum levels in stable COPD were increased compared to healthy non-smokers, but during an acute exacerbation, levels were lower [69].
- Levels of serum H₂S correlated positively with lung function (FEV₁ % predicted) [83].

COPD: Chronic obstructive pulmonary disease; H₂S: Hydrogen sulfide.

study using gas chromatography, the levels of H₂S in exhaled breath in lung transplant recipients was not influenced by the presence of obliterative bronchiolitis, a sign of chronic allograft rejection [85]. Finally, Toombs et al. found a level of 6.4 ppb in exhaled air of normal volunteers which increased transiently to 26.9 ppb following a rapid infusion of sodium sulfite preparation of 0.15–0.2 mg/kg [86]. H₂S was measured on-line by two sulfide detectors that contained two different sensing elements that had a broad analytical range of 10 ppb to 5 ppm. In another study, the levels of sulfides in exhaled breath between normal volunteers and those with cystic fibrosis were measured using ultra-trace gas chromatography. They found that only the control net uptake of carbonyl sulfide but not of dimethyl sulfide or carbon disulfide was less negative in patients with cystic fibrosis (–110 parts per trillion by volume) compared with normal volunteers (–250 parts per trillion) [87].

Thus, measuring H₂S in exhaled breath is feasible and because exhaled NO has proven to be a useful biomarker of asthma for clinical practice, H₂S in exhaled breath may indicate a specific phenotype of asthma. However, there are potentially many sources of H₂S that could account for the levels in exhaled breath and these include periodontal disease [88–90], the gut bacterial flora [91,92] and tissues in the conducting airways and of the gut that contains CSE and CBS enzymes [12]. How much bacteria as opposed to human sources produce H₂S is not known. Conditions such as COPD associated with systemic or localized tissue inflammation can be lead to the activation of H₂S-producing enzymes.

Cross-talk between H₂S & NO

Since both NO and H₂S are elevated in the airways of asthma, one could speculate on the potential interactions between NO and H₂S. In fact, a negative correlation was found between sputum H₂S and exhaled NO levels in asthmatic subjects [77]. There is evidence of interaction between H₂S and NO leading to the formation of nitrosothiol, which may then regulate the effects of both gasotransmitters [49]. S-nitrosothiols signaling may be involved in the regulation of ventilation-perfusion

matching and in baseline airway tone [93], and GSNO has been shown to have bronchodilator properties [94]. There are also increased levels of nitrosothiols in exhaled breath condensates in inflammatory airway diseases such as asthma [95]. On the other hand, H₂S can directly inhibit nitric oxide synthase activity and NO production [24,96]. Conversely, NO donors upregulate H₂S production in rat vascular tissues [97].

Conclusion

The importance of H₂S as a third gasotransmitter is gathering pace and its intracellular properties being elucidated. However, how it acts and how it is regulated and what its physiologic function in the lungs is remain unclear. When given exogenously, H₂S can have anti-inflammatory and anti-fibrotic effects. However, it can have opposite effects under certain conditions. It appears to be elevated in asthma, and could be a biomarker of neutrophilic-type asthma. Certainly, further work is needed to confirm this, and measurement in exhaled breath may represent a more accurate way of measuring it. Levels in exhaled breath may represent contribution from bacterial colonization, the effect of environmental exposures and the lung inflammatory process. H₂S may have therapeutic uses but this must be approached cautiously since it has 'the good and the bad, the ugly and the promising' actions [15].

Expert commentary

H₂S has been proposed to act as a gasotransmitter at the cellular level. Current evidence suggests that H₂S can inhibit cellular activation and inflammation, cell proliferation and reduce

fibrotic processes and is a relaxant of airway and vascular smooth muscle. H₂S has been implicated as a sensor of hypoxia. It acts as a reducing agent and induces sulfhydrylation of various proteins leading to functional changes. It activates neurogenic inflammation. Further research is needed to define its physiology in the lungs and its therapeutic possibility.

Five-year view

There will be an increased interest in the cellular provenance of H₂S and how it is regulated in lung cells. With better assays that will become available for H₂S in small quantities, the role of endogenously produced H₂S will become clearer both in normal and disease states. Assays for directly measuring H₂S in exhaled breath will provide an indication as a biomarker of disease particularly in asthma or COPD. Since one source of H₂S is bacterial, it may be an indicator of such infections in the airways or lungs. New methods of delivering H₂S or increasing the production of H₂S at sites of lung injury or inflammation could lead to a new form of treatment.

Financial & competing interests disclosure

This work was supported by the Wellcome Trust and MRC, and by the NIHR Respiratory Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Hydrogen sulfide (H₂S) is produced through the actions of enzymes, cystathionine-β-synthase, cystathionine-γ-lyase and 3-mercaptopyruvate sulfurtransferase, via the metabolism of cysteine, homocysteine and cystathionine. The contribution of each of these enzymes is dependent on the cell type and the cell milieu.
- H₂S can suppress inflammation through an action on inflammatory cells, although it enhances neurogenic inflammation.
- H₂S can relax airway and vascular smooth muscle cells, mechanisms that could involve ATP-sensitive K⁺ channels. It has been implicated in hypoxic pulmonary vasoconstriction and as an oxygen sensor.
- Exogenous H₂S inhibited allergen-induced eosinophilic inflammation and remodeling in mouse asthma models. H₂S also inhibited human airway smooth muscle proliferation and cytokine release.
- H₂S has been detected in plasma and sputum samples, and in asthma, levels in induced sputum were correlated with obstructive neutrophilic asthma. However, these levels may be spuriously high, reflecting total sulfide.

References

Papers of special note have been highlighted as:

- of interest
- of considerable interest

- 1 Gibeon D, Chung KF. The investigation of severe asthma to define phenotypes. *Clin. Exp. Allergy* 42(5), 678–692 (2012).
- 2 McGrath KW, Icitovic N, Boushey HA *et al.* A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. *Am. J. Respir. Crit. Care Med.* 185(6), 612–619 (2012).
- 3 Haldar P, Pavord ID, Shaw DE *et al.* Cluster analysis and clinical asthma phenotypes. *Am. J. Respir. Crit. Care Med.* 178(3), 218–224 (2008).
- 4 Woodruff PG, Modrek B, Choy DF *et al.* T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am. J. Respir. Crit. Care Med.* 180(5), 388–395 (2009).
- 5 Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophilic inflammation in severe persistent asthma. *Am. J. Respir. Crit. Care Med.* 160(5 Pt 1), 1532–1539 (1999).
- 6 Chung KF, Godard P, Adelroth E *et al.* Difficult/therapy-resistant asthma: the need for an integrated approach to define clinical phenotypes, evaluate risk factors, understand pathophysiology and find novel therapies. ERS Task Force on Difficult/Therapy-

- Resistant Asthma. *Eur. Respir. J.* 13(5), 1198–1208 (1999).
- 7 Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. American Thoracic Society. *Am. J. Respir. Crit. Care Med.* 162(6), 2341–2351 (2000).
 - 8 Nair P, Gaga M, Zervas E *et al.* Safety and efficacy of a CXCR2 antagonist in patients with severe asthma and sputum neutrophils: a randomized, placebo controlled clinical trial. *Clin. Exp. Allergy* (2012) (In Press).
 - 9 Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat. Med.* 18(5), 716–725 (2012).
 - **Description of approaches to molecular phenotyping of asthma.**
 - 10 Jia G, Erickson RW, Choy DF *et al.* Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J. Allergy. Clin. Immunol.* 130(3), 647–654, e10 (2012).
 - 11 Dweik RA, Boggs PB, Erzurum SC *et al.* An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am. J. Respir. Crit. Care Med.* 184(5), 602–615 (2011).
 - 12 Wang R. Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. *Physiol. Rev.* 92(2), 791–896 (2012).
 - **Up-to-date review of the physiology and pathophysiology of hydrogen sulfide (H₂S) looking at all biological systems.**
 - 13 Wang R. Shared signaling pathways among gasotransmitters. *Proc. Natl Acad. Sci. USA* 109(23), 8801–8802 (2012).
 - 14 Szabo C. Hydrogen sulphide and its therapeutic potential. *Nat. Rev. Drug Discov.* 6(11), 917–935 (2007).
 - 15 Whiteman M, Le Trionnaire S, Chopra M, Fox B, Whatmore J. Emerging role of hydrogen sulfide in health and disease: critical appraisal of biomarkers and pharmacological tools. *Clin. Sci.* 121(11), 459–488 (2011).
 - **Excellent review of the pharmacological tools available to study the effects and roles of H₂S.**
 - 16 Shibuya N, Mikami Y, Kimura Y, Nagahara N, Kimura H. Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces hydrogen sulfide. *J. Biochem.* 146(5), 623–626 (2009).
 - 17 Shibuya N, Tanaka M, Yoshida M *et al.* 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid. Redox Signal.* 11(4), 703–714 (2009).
 - 18 Kabil O, Banerjee R. Redox biochemistry of hydrogen sulfide. *J. Biol. Chem.* 285(29), 21903–21907 (2010).
 - 19 Mikami Y, Shibuya N, Kimura Y, Nagahara N, Ogasawara Y, Kimura H. Thioredoxin and dihydrolipoic acid are required for 3-mercaptopyruvate sulfurtransferase to produce hydrogen sulfide. *Biochem. J.* 439(3), 479–485 (2011).
 - 20 Olson KR, Whitfield NL. Hydrogen sulfide and oxygen sensing in the cardiovascular system. *Antioxid. Redox Signal.* 12(10), 1219–1234 (2010).
 - **Describes the important role of H₂S in oxygen sensing.**
 - 21 Baskar R, Li L, Moore PK. Hydrogen sulfide induces DNA damage and changes in apoptotic gene expression in human lung fibroblast cells. *FASEB J.* 21(1), 247–255 (2007).
 - 22 Perry MM, Hui CK, Whiteman M *et al.* Hydrogen sulfide inhibits proliferation and release of IL-8 from human airway smooth muscle cells. *Am. J. Respir. Cell. Mol. Biol.* 45(4), 746–752 (2011).
 - **Description of the role of H₂S in human airway smooth muscle.**
 - 23 Wang P, Zhang G, Wondimu T, Ross B, Wang R. Hydrogen sulfide and asthma. *Exp. Physiol.* 96(9), 847–852 (2011).
 - 24 Zhu XY, Liu SJ, Liu YJ, Wang S, Ni X. Glucocorticoids suppress cystathionine gamma-lyase expression and H₂S production in lipopolysaccharide-treated macrophages. *Cell Mol. Life Sci.* 67(7), 1119–1132 (2010).
 - 25 Zano RD, Brancalione V, Distrutti E, Fiorucci S, Cirino G, Wallace JL. Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation. *FASEB J.* 20(12), 2118–2120 (2006).
 - 26 Ekundi-Valentim E, Santos KT, Camargo EA *et al.* Differing effects of exogenous and endogenous hydrogen sulphide in carrageenan-induced knee joint synovitis in the rat. *Br. J. Pharmacol.* 159(7), 1463–1474 (2010).
 - 27 Li L, Fox B, Keeble J, Salto-Tellez M *et al.* The complex effects of the slow-releasing hydrogen sulfide donor GYY4137 in a model of acute joint inflammation and in human cartilage cells. *J. Cell. Mol. Med.* 17(3), 365–376 (2013).
 - 28 Bucci M, Papapetropoulos A, Vellecco V *et al.* Hydrogen sulfide is an endogenous inhibitor of phosphodiesterase activity. *Arterioscler. Thromb. Vasc. Biol.* 30(10), 1998–2004 (2010).
 - 29 Whiteman M, Li L, Kostetski I *et al.* Evidence for the formation of a novel nitrosothiol from the gaseous mediators nitric oxide and hydrogen sulphide. *Biochem. Biophys. Res. Commun.* 343(1), 303–310 (2006).
 - 30 Whiteman M, Armstrong JS, Chu SH *et al.* The novel neuromodulator hydrogen sulfide: an endogenous peroxynitrite ‘scavenger’? *J. Neurochem.* 90(3), 765–768 (2004).
 - 31 Fiorucci S, Orlandi S, Mencarelli A *et al.* Enhanced activity of a hydrogen sulphide-releasing derivative of mesalamine (ATB-429) in a mouse model of colitis. *Br. J. Pharmacol.* 150(8), 996–1002 (2007).
 - 32 Whiteman M, Li L, Rose P, Tan CH, Parkinson DB, Moore PK. The effect of hydrogen sulfide donors on lipopolysaccharide-induced formation of inflammatory mediators in macrophages. *Antioxid. Redox Signal.* 12(10), 1147–1154 (2010).
 - 33 Liu H, Bai XB, Shi S, Cao YX. Hydrogen sulfide protects from intestinal ischaemia-reperfusion injury in rats. *J. Pharm. Pharmacol.* 61(2), 207–212 (2009).
 - 34 Tyagi N, Moshal KS, Sen U *et al.* H₂S protects against methionine-induced oxidative stress in brain endothelial cells. *Antioxid. Redox Signal.* 11(1), 25–33 (2009).
 - 35 Xu ZS, Wang XY, Xiao DM *et al.* Hydrogen sulfide protects MC3T3-E1 osteoblastic cells against H₂O₂-induced oxidative damage-implications for the treatment of osteoporosis. *Free Radic. Biol. Med.* 50(10), 1314–1323 (2011).
 - 36 Mariggio MA, Minunno V, Riccardi S, Santacrose R, De Rinaldis P, Fumarulo R. Sulfide enhancement of PMN apoptosis. *Immunopharmacol. Immunotoxicol.* 20(3), 399–408 (1998).
 - 37 Dufton N, Natividad J, Verdu EF, Wallace JL. Hydrogen sulfide and resolution of acute inflammation: A comparative study utilizing a novel fluorescent probe. *Sci. Rep.* 2, 499 (2012).
 - 38 Chen YH, Wu R, Geng B *et al.* Endogenous hydrogen sulfide reduces airway inflammation and remodeling in a rat model of asthma. *Cytokine* 45(2), 117–123 (2009).

- 39 Benetti LR, Campos D, Gurgueira SA *et al.* Hydrogen sulfide inhibits oxidative stress in lungs from allergic mice in vivo. *Eur. J. Pharmacol.* 698(1–3), 463–469 (2013).
- 40 Chen YH, Wang PP, Wang XM *et al.* Involvement of endogenous hydrogen sulfide in cigarette smoke-induced changes in airway responsiveness and inflammation of rat lung. *Cytokine* 53(3), 334–341 (2011).
- 41 Han W, Dong Z, Dimitropoulou C, Su Y. Hydrogen sulfide ameliorates tobacco smoke-induced oxidative stress and emphysema in mice. *Antioxid. Redox Signal.* 15(8), 2121–2134 (2011).
- 42 Wang T, Wang L, Zaidi SR *et al.* Hydrogen sulfide attenuates particulate matter-induced human lung endothelial barrier disruption via combined reactive oxygen species scavenging and Akt activation. *Am. J. Respir. Cell. Mol. Biol.* 47(4), 491–496 (2012).
- Describes the potential protective role for H₂S against air pollution.
- 43 Zhang H, Zhi L, Moochhala S, Moore PK, Bhatia M. Hydrogen sulfide acts as an inflammatory mediator in cecal ligation and puncture-induced sepsis in mice by upregulating the production of cytokines and chemokines via NF-kappaB. *American journal of physiology Lung Cell. Mol. Physiol.* 292(4), L960–L971 (2007).
- 44 Li L, Bhatia M, Zhu YZ *et al.* Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse. *FASEB J.* 19(9), 1196–1198 (2005).
- 45 Hui Y, Du J, Tang C, Bin G, Jiang H. Changes in arterial hydrogen sulfide (H₂S) content during septic shock and endotoxin shock in rats. *J. Infect.* 47(2), 155–160 (2003).
- 46 Ang SF, Moochhala SM, Bhatia M. Hydrogen sulfide promotes transient receptor potential vanilloid 1-mediated neurogenic inflammation in polymicrobial sepsis. *Crit. Care Med.* 38(2), 619–628 (2010).
- 47 Trevisani M, Patacchini R, Nicoletti P *et al.* Hydrogen sulfide causes vanilloid receptor 1-mediated neurogenic inflammation in the airways. *Br. J. Pharmacol.* 145(8), 1123–1131 (2005).
- Describes the potential for H₂S in mediating neurogenic inflammation.
- 48 Bhatia M, Zhi L, Zhang H, Ng SW, Moore PK. Role of substance P in hydrogen sulfide-induced pulmonary inflammation in mice. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 291(5), L896–L904 (2006).
- 49 Whiteman M, Li L, Kostetski I *et al.* Evidence for the formation of a novel nitrosothiol from the gaseous mediators nitric oxide and hydrogen sulphide. *Biochem. Biophys. Res. Commun.* 343(1), 303–310 (2006).
- 50 Gorg B, Foster N, Reinehr R *et al.* Benzodiazepine-induced protein tyrosine nitration in rat astrocytes. *Hepatology* 37(2), 334–342 (2003).
- 51 Wei HL, Zhang CY, Jin HF, Tang CS, Du JB. Hydrogen sulfide regulates lung tissue-oxidized glutathione and total antioxidant capacity in hypoxic pulmonary hypertensive rats. *Acta Pharmacol. Sin.* 29(6), 670–679 (2008).
- 52 Chung KF, Marwick JA. Molecular mechanisms of oxidative stress in airways and lungs with reference to asthma and chronic obstructive pulmonary disease. *Ann. NY Acad. Sci.* 1203, 85–91 (2010).
- 53 Michaeloudes C, Chang PJ, Petrou M, Chung KF. Transforming growth factor-beta and nuclear factor E2-related factor 2 regulate antioxidant responses in airway smooth muscle cells: role in asthma. *Am. J. Respir. Crit. Care Med.* 184(8), 894–903 (2011).
- 54 Hayes JD, Ashford ML. Nrf2 orchestrates fuel partitioning for cell proliferation. *Cell Metab.* 16(2), 139–141 (2012).
- 55 Yang G, Li H, Tang G *et al.* Increased neointimal formation in cystathionine gamma-lyase deficient mice: role of hydrogen sulfide in alpha5beta1-integrin and matrix metalloproteinase-2 expression in smooth muscle cells. *J. Mol. Cell. Cardiol.* 52(3), 677–688 (2012).
- 56 Mustafa AK, Gadalla MM, Sen N *et al.* H₂S signals through protein S-sulphydration. *Sci. Signal.* 2(96), ra72 (2009).
- 57 Yang G, Wu L, Wang R. Pro-apoptotic effect of endogenous H₂S on human aorta smooth muscle cells. *FASEB J.* 20(3), 553–555 (2006).
- 58 Yang G, Wu L, Bryan S, Khaper N, Mani S, Wang R. Cystathionine gamma-lyase deficiency and overproliferation of smooth muscle cells. *Cardiovasc. Res.* 86(3), 487–495 (2010).
- 59 Fang L, Li H, Tang C, Geng B, Qi Y, Liu X. Hydrogen sulfide attenuates the pathogenesis of pulmonary fibrosis induced by bleomycin in rats. *Can. J. Physiol. Pharmacol.* 87(7), 531–538 (2009).
- 60 Kubo S, Doe I, Kurokawa Y, Kawabata A. Hydrogen sulfide causes relaxation in mouse bronchial smooth muscle. *J. Pharmacol. Sci.* 104(4), 392–396 (2007).
- 61 Lim JJ, Liu YH, Khin ES, Bian JS. Vasoconstrictive effect of hydrogen sulfide involves downregulation of cAMP in vascular smooth muscle cells. *Am. J. Physiol. Cell. Physiol.* 295(5), C1261–C1270 (2008).
- 62 Derwall M, Francis RC, Kida K *et al.* Administration of hydrogen sulfide via extracorporeal membrane lung ventilation in sheep with partial cardiopulmonary bypass perfusion: a proof of concept study on metabolic and vasomotor effects. *Crit. Care* 15(1), R51 (2011).
- 63 Olson KR, Whitfield NL, Bearden SE *et al.* Hypoxic pulmonary vasodilation: a paradigm shift with a hydrogen sulfide mechanism. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 298(1), R51–R60 (2010).
- 64 Prabhakar NR. Hydrogen sulfide (H₂S): a physiologic mediator of carotid body response to hypoxia. *Adv. Exp. Med. Biol.* 758, 109–113 (2012).
- 65 Evans AM, Hardie DG, Peers C, Mahmoud A. Hypoxic pulmonary vasoconstriction: mechanisms of oxygen-sensing. *Curr. Opin. Anaesthesiol.* 24(1), 13–20 (2011).
- 66 Modis K, Coletta C, Erdelyi K, Papapetropoulos A, Szabo C. Intramitochondrial hydrogen sulfide production by 3-mercaptopyruvate sulfurtransferase maintains mitochondrial electron flow and supports cellular bioenergetics. *FASEB J.* 27(2), 601–611 (2013).
- 67 Fu M, Zhang W, Wu L, Yang G, Li H, Wang R. Hydrogen sulfide (H₂S) metabolism in mitochondria and its regulatory role in energy production. *Proc. Natl Acad. Sci. USA* 109(8), 2943–2948 (2012).
- One of the first studies to implicate a role for H₂S in mitochondrial energetics.
- 68 Hyspler R, Ticha A, Indrova M *et al.* A simple, optimized method for the determination of sulphide in whole blood by GC-mS as a marker of bowel fermentation processes. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 770(1–2), 255–259 (2002).
- 69 Richardson CJ, Magee EA, Cummings JH. A new method for the determination of sulphide in gastrointestinal contents and whole blood by microdistillation and ion chromatography. *Clin. Chim. Acta* 293(1–2), 115–125 (2000).

- 70 Chen YH, Yao WZ, Geng B *et al.* Endogenous hydrogen sulfide in patients with COPD. *Chest* 128(5), 3205–3211 (2005).
- 71 Chen YH, Yao WZ, Gao JZ, Geng B, Wang PP, Tang CS. Serum hydrogen sulfide as a novel marker predicting bacterial involvement in patients with community-acquired lower respiratory tract infections. *Respirology* 14(5), 746–752 (2009).
- 72 Whitfield NL, Kreimier EL, Verdial FC, Skovgaard N, Olson KR. Reappraisal of H₂S/sulfide concentration in vertebrate blood and its potential significance in ischemic preconditioning and vascular signaling. *Am. J. Physiol. RegulIntegr. Comp. Physiol.* 294(6), R1930–R1937 (2008).
- 73 Furne J, Saeed A, Levitt MD. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. *Am. J. Physiol. RegulIntegr. Comp. Physiol.* 295(5), R1479–R1485 (2008).
- 74 Levitt MD, Abdel-Rehim MS, Furne J. Free and acid-labile hydrogen sulfide concentrations in mouse tissues: anomalously high free hydrogen sulfide in aortic tissue. *Antioxid. Redox Signal.* 15(2), 373–378 (2011).
- 75 Wu R, Yao WZ, Chen YH, Geng B, Tang CS. [Plasma level of endogenous hydrogen sulfide in patients with acute asthma]. *Beijing Da Xue Xue Bao* 40(5), 505–508 (2008).
- 76 Tian M, Wang Y, Lu YQ, Yan M, Jiang YH, Zhao DY. Correlation between serum H₂S and pulmonary function in children with bronchial asthma. *Mol. Med. Report.* 6(2), 335–358 (2012).
- 77 Saito J, Zhang Q, Hui C *et al.* Sputum hydrogen sulfide as a novel biomarker of obstructive neutrophilic asthma. *J. Allergy Clin. Immunol.* 131(1), 232–234 (2013).
- 78 Li L, Whiteman M, Moore PK. Dexamethasone inhibits lipopolysaccharide-induced hydrogen sulphide biosynthesis in intact cells and in an animal model of endotoxic shock. *J. Cell. Mol. Med.* 13(8b), 2684–2692 (2009).
- 79 Hilty M, Burke C, Pedro H *et al.* Disordered microbial communities in asthmatic airways. *PLoS ONE* 5(1), e8578 (2010).
- 80 Huang YJ, Nelson CE, Brodie EL *et al.* Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. *J. Allergy Clin. Immunol.* 127(2), 372–381 (2011).
- 81 Shatalin K, Shatalina E, Mironov A, Nudler E. H₂S: a universal defense against antibiotics in bacteria. *Science* 334(6058), 986–990 (2011).
- 82 Chung KF, Adcock IM. Multifaceted mechanisms in COPD: inflammation, immunity, and tissue repair and destruction. *Eur. Respir. J.* 31(6), 1334–1356 (2008).
- 83 Chen YH, Yao WZ, Ding YL, Geng B, Lu M, Tang CS. Effect of theophylline on endogenous hydrogen sulfide production in patients with COPD. *Pulm. Pharmacol. Ther.* 21(1), 40–46 (2008).
- 84 Morselli-Labate AM, Fantini L, Pezzilli R. Hydrogen sulfide, nitric oxide and a molecular mass 66 u substance in the exhaled breath of chronic pancreatitis patients. *Pancreatolgy* 7(5–6), 497–504 (2007).
- 85 Studer SM, Orens JB, Rosas I *et al.* Patterns and significance of exhaled-breath biomarkers in lung transplant recipients with acute allograft rejection. *J. Heart Lung Transplantation* 20(11), 1158–1166 (2001).
- 86 Toombs CF, Insko MA, Wintner EA *et al.* Detection of exhaled hydrogen sulphide gas in healthy human volunteers during intravenous administration of sodium sulphide. *Br. J. Clin. Pharmacol.* 69(6), 626–636 (2010).
- **Reported measurement of H₂S in exhaled breath.**
- 87 Kamboures MA, Blake DR, Cooper DM *et al.* Breath sulfides and pulmonary function in cystic fibrosis. *Proc. Natl Acad. Sci. USA* 102(44), 15762–15767 (2005).
- 88 Nakano Y, Yoshimura M, Koga T. Correlation between oral malodor and periodontal bacteria. *Microbes Infect.* 4(6), 679–683 (2002).
- 89 Morita M, Wang HL. Relationship between sulcular sulfide level and oral malodor in subjects with periodontal disease. *J Periodontol.* 72(1), 79–84.
- 90 Lee H, Kho HS, Chung JW, Chung SC, Kim YK. Volatile sulfur compounds produced by *Helicobacter pylori*. *J. Clin. Gastroenterol.* 40(5), 421–426 (2006).
- 91 Picton R, Eggo MC, Merrill GA, Langman MJ, Singh S. Mucosal protection against sulphide: importance of the enzyme rhodanese. *Gut* 50(2), 201–205 (2002).
- 92 Rowan FE, Docherty NG, Coffey JC, O'Connell PR. Sulphate-reducing bacteria and hydrogen sulphide in the aetiology of ulcerative colitis. *Br. J. Surg.* 96(2), 151–158 (2009).
- 93 Gaston B, Singel D, Doctor A, Stamler JS. S-nitrosothiol signaling in respiratory biology. *Am. J. Respir. Crit. Care Med.* 173(11), 1186–1193 (2006).
- 94 Que LG, Liu L, Yan Y *et al.* Protection from experimental asthma by an endogenous bronchodilator. *Science* 308(5728), 1618–1621 (2005).
- 95 Corradi M, Montuschi P, Donnelly LE, Pesci A, Kharitonov SA, Barnes PJ. Increased nitrosothiols in exhaled breath condensate in inflammatory airway diseases. *Am. J. Respir. Crit. Care Med.* 163(4), 854–858 (2001).
- 96 Kubo S, Kurokawa Y, Doe I, Masuko T, Sekiguchi F, Kawabata A. Hydrogen sulfide inhibits activity of three isoforms of recombinant nitric oxide synthase. *Toxicology* 241(1–2), 92–97 (2007).
- 97 Yanfei W, Lin S, Junbao D, Chaoshu T. Impact of L-arginine on hydrogen sulfide/cystathionine-gamma-lyase pathway in rats with high blood flow-induced pulmonary hypertension. *Biochem. Biophys. Res. Commun.* 345(2), 851–857 (2006).

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