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A timeline of hydrogen sulfide (H₂S) research: from environmental toxin to biological mediator

Csaba Szabo

Department of Anesthesiology, University of Texas Medical Branch, Galveston, Texas, United States of America

Abstract

The history of H₂S - as an environmental toxin - dates back to 1700, to the observations of the Italian physician Bernardino Ramazzini, whose book “De Morbis Artificum Diatriba” described the painful eye irritation and inflammation of “sewer gas” in sewer workers. The gas has subsequently been identified as hydrogen sulfide (H₂S), and opened three centuries of research into the biological roles of H₂S. The current article highlights the key discoveries in the field of H₂S research, including (a) the toxicological studies, which characterized H₂S as an environmental toxin, and identified some of its modes of action, including the inhibition of mitochondrial respiration; (b) work in the field of bacteriology, which, starting in the early 1900’s, identified H₂S as a bacterial product - with subsequently defined roles in the regulation of periodontal disease (oral bacterial flora), intestinal epithelial cell function (enteral bacterial flora) as well as in the regulation of bacterial resistance to antibiotics; and (c), work in diverse fields of mammalian biology, which, starting in the 1940’s, identified H₂S as an endogenous mammalian enzymatic product, the functions of which - among others, in the cardiovascular and nervous system - have become subjects of intensive investigation for the last decade. The current review not only enumerates the key discoveries related to H₂S made over the last three centuries, but also compiles the most frequently cited papers in the field which have been published over the last decade and highlights some of the current ‘hot topics’ in the field of H₂S biology.

Graphical Abstract

Address for correspondence: Csaba Szabo M.D., Ph.D., Department of Anesthesiology, University of Texas Medical Branch, Galveston, Texas, 301 University Boulevard, Galveston, TX 77555., szabocsaba@aol.com.

Author Disclosure Statement

C.S. is a founder, officer and shareholder of CBS Therapeutics Inc., an UTMB spin-off company focusing on therapeutic approaches around H₂S biosynthesis inhibition in cancer cells.

Author contributions

CS – conceived the topic, researched the literature, interviewed - via email - several investigators working in the field of H₂S, and wrote the paper.

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Keywords

gasotransmitters; nitric oxide; cystathionine- β -synthase; cystathionine gamma lyase; 3-mercaptopyruvate sulfurtransferase

“Those who cannot learn from history are doomed to repeat it.”

(George Santayana)

1. H₂S as an environmental toxin

The history of H₂S - as an environmental toxin - dates back to 1700, to the observations of the Italian physician Bernardino Ramazzini (1633-1714), whose book “De Morbis Artificum Diatriba” [1] is the reason why he is widely considered the founding father of the discipline of occupational medicine¹ [2]. The book did not gain a wide audience until it was translated to English and published in 1940 [3]. In a chapter entitled “Diseases of Cleaners of Privies and Cesspits” he described the painful irritation and inflammation of “sewer gas” on the eye in sewer workers. He noted that this inflammation can lead to subsequent bacterial infections, and that it can result in very severe health effects including blindness. Although Ramazzini was not aware of the chemical nature of the species responsible, with remarkable insight, Ramazzini hypothesized that when the cleaners disturbed the excrement during their work, an unknown volatile acid was produced, which was the cause of the adverse ocular effects. He also, correctly, predicted that this gas may also damage the lungs,² although in his book he did not report any pulmonary pathologies in the workers he examined. He also surmised that this species may have been also responsible for the pungent smell of the excrement. Ramazzini also noted that copper coins in the workers’ pockets turn black on their surfaces, and hypothesized that this effect is due to the same, acidic, gaseous substance that is also causing the adverse effects on the eye. With this amazing insight, Ramazzini can be credited as the first scientist not only to propose H₂S/copper chemistry in specific,³ but also to be a pioneer of three centuries of subsequent H₂S research (Table 1) in general.

¹Hippocrates’ counsel (“When you come to a patient’s house, you should ask him what sort of pain he has, what caused them, how many days he has been ill, whether the bowels are working and what sort of food he eats”), was extended by Ramazzini as follows: “I may venture to add one more question: What occupation does he follow?”

²“I am inclined to think that some volatile acid is given off by this camerine of filth when workers disturb it... such effluvia ought, one would think, to impair the lungs. Nevertheless, it is only against the eyes that these foul exhalations wage ruthless war, and they attack them so cruelly with their piercing stings that they rob them of life...”

In the late 1770's Parisians experienced many accidents due to a gas emanating from its antiquated sewer system. A commission was appointed to study the causes of the accidents. Their report, published in 1785, described two types of poisonings: a mild form (affecting the eye, in line with Ramazzini's observations) which they called "*mitte*" and a severe form of rapid, severe asphyxia which they designated as "*plomb*". Although the initial report did not make the connection to H₂S gas, subsequent reports did, and the concept of H₂S-associated intoxication was born. It is likely that these public observations inspired the, vivid description of the ill effect of sewer gas (produced in the ancient sewer systems of Paris) of Victor Hugo in his classic book *Les Misérables* (1862). Hugo referred to the Parisian sewers as "the intestine of the Leviathan." The presence of H₂S gas in sewers was subsequently directly confirmed by Dupuytren in 1806 [6].

As a pure chemical species, the discovery of H₂S (1775) is credited to Swedish-German chemist Carl Wilhelm Scheele (1742-1786), who produced it through the reaction of acid with metal (poly)sulfides or by heating sulfur in hydrogen gas.⁴ In 1776, the chemical composition of the gas was determined by the French chemist Claude Louis Berthollet (1789) who also noted its acidic chemical nature [7].

The first biological experiment (of which the records are available) that directly investigated the effect of pure H₂S in animals was published in 1803 by French anatomist Francois Chaussier (1746-1828) [8]. He described the lethal effects of H₂S gas, when the gas was absorbed through the skin, while the horse was allowed to breathe fresh air. He also documented adverse effects of H₂S-containing solutions when administered directly into the stomach or into the rectum. Subsequent studies used injections of H₂S-saturated solutions, and reported respiratory effects (excitation, deep breathing), convulsive movements, and, at high doses, asphyxia and death. Other studies, after injection of H₂S-containing solutions into the venous system of animals, reported that some of the gas is exiting the animal through exhalation via the lung. Some of these early studies have also noted a change in blood color, an effect, which was subsequently attributed to the reaction of H₂S with hemoglobin, producing sulfhemoglobinemia (the latter term was coined by Hoppe-Seyler in 1863, who also characterized the absorption spectrum of hemoglobin) [reviewed in: 7].

Over the subsequent 150 years, generations of toxicological researchers have investigated the toxicological effects of H₂S in various species including human. The results of these studies are now summarized in comprehensive monographs, including limits and methods of its detection, occupational exposure limits, and the multiple biological effects of H₂S on various cells and organs. [reviewed in: 7,9–15]. Among the more recent studies, the work of Attene-Ramos should be mentioned, who demonstrated the genotoxic effect of high doses of H₂S [16]. Nicholson [17], Khan [18] and later Dorman and colleagues [19] have directly demonstrated the inhibition of cytochrome c oxidase activity *ex vivo* in tissues after H₂S

³Subsequent work revealed that the copper-H₂S reaction has multiple biological implications. For instance, in biological contexts, the binding of H₂S to copper plays a key role in the H₂S-mediated inhibition of mitochondrial Complex IV [4] (see, for more detail, below), and H₂S-copper reactions are responsible for the sensitive detection of H₂S by the olfactory nerves [5].

⁴As a follower of the erroneous "phlogiston theory", which was dominant in his time, he considered it a combination of sulfur, phlogiston and heat. (Through separate lines of work, Scheele is also credited as the co-discoverer of oxygen or "fire air" as well as the elements chlorine and manganese.)

exposure of experimental animals, and implicating these effects in the disruption of respiratory and mitochondrial functions in the mammalian brain (and other tissues) after H₂S exposure *in vivo*. Dorman and colleagues have also conducted comprehensive, state-of-the-art toxicology evaluation of H₂S inhalation in various animal species, as well as provided the first microarray analysis demonstrating that H₂S has wide-ranging effects on gene expression (associated with a variety of biological processes including cell cycle regulation, cellular division, DNA metabolism and repair, protein kinase regulation, cytoskeletal organization and biogenesis). [20,21].

As far as the mode of H₂S' toxic action, the initial theory focusing on the formation of sulfhemoglobin in blood, was subsequently put into question, when it was noted that patients with H₂S poisoning do not exhibit sulfhemoglobinemia; later on, even the formation of sulfhemoglobin was called into question [reviewed in: 7]. It is currently accepted that H₂S exerts its toxicological actions on the molecular level primarily through the inhibition of mitochondrial respiration, via inhibition of mitochondrial Complex IV (cytochrome c oxidase). Via this action, the consumption of O₂ is inhibited and mitochondrial electron transport and ATP generation is blocked. Nervous and cardiac tissues, which have the highest oxygen demand, are especially sensitive to the disruption of oxidative metabolism [22]. The first precursor of this theory can be attributed to Kaufmann and Rosenthal in 1865, who proposed that H₂S poisoning induces "oxygen hunger" and that H₂S poisoning has similarities to suffocation [23]. The fact that H₂S is an inhibitor of cytochrome c oxidase was already known in the 1940's [24]; it has been further characterized by Chance in 1965 [25] and subsequently thoroughly characterized through three decades of work by Nichols, starting in 1975 [26,27]. However - as demonstrated through the work of dozens of investigators over the last five decades - the toxicological mode of H₂S' action is definitely more complex, as H₂S is capable of interacting with multiple intra- and extracellular proteins, via a variety of actions (including sulphydration reactions, see below); these actions are likely to mediate (or modulate) the toxicological action of this gas.

Although the toxic/metabolism-suppressing effects of H₂S has many aspects, we would like to emphasize one particular discovery that generated a lot of attention and contributed to the expansion and further revitalization of the field of H₂S research over the last decade. The subject relates to the concept of "H₂S-induced, on-demand suspended animation" (or hibernation). In a short article in Science, in 2005, Roth and colleagues from the Fred Hutchinson Cancer Center in Seattle reported that "*H₂S induces a suspended animation-like state in mice*" [28]. In this study, mice, subjected to H₂S inhalation were shown to develop a "hibernation-like state". When placed in an atmosphere of 20–80 ppm H₂S gas, mice exhibited dose-dependent reductions in core body temperature and metabolic rate. Over the course of several hours of H₂S exposure, the animals' output (down to 10% of metabolic rate continued to decrease, as measured by their CO₂ baseline). When the chamber of the animals was cooled, body temperature reached as low as 15 °C. These effects were found reversible after resuscitation at room air and warming of the chambers. The original "hibernation" studies were subsequently reproduced by other groups and it was suggested that some of the H₂S-induced cardiovascular responses (e.g. decreased heart rate) may be consistent with the physiology of hibernation [29,30]. Although subsequent studies have indicated that (a) the metabolic effects are only minimal or nonexistent in larger animals and

(b) a decreased *physical activity* of the mice (and the consequently decreased skeletal muscle-related energy consumption) is a significant contributor to the hibernation-like effects of H₂S inhalation in conscious mice [31], the study by Roth and colleagues has attracted significant attention to multiple facets of H₂S biology and has stimulated multiple lines of present-day work aimed to explore the potential beneficial and therapeutic effects of H₂S donation (see also below in more detail).

2. H₂S as an endogenous product in bacteria, plants and other non-mammalian species

The history of H₂S - as a *bacterial product* - dates back to 1877 [32], to the work of French microbiologist Ulysse Gayon (1845–1919) who noticed the production of H₂S by microbes in spoiled chicken eggs and described the use of paper strips impregnated with lead acetate for detecting the presence of this gaseous byproduct.⁵ In subsequent studies, Beijerinck [35], Schardinger [36] and Durham [37] described the production of H₂S by putrefactive organisms associated with soil and feces. For example, in 1894, Schardinger observed that when water polluted with fecal material was added to peptone solution and incubated, it produces a characteristic odor, and that it blackens a strip of lead acetate paper which was suspended over the liquid [36].

In the subsequent 100 years, the formation of H₂S has been confirmed in a large number of bacterial species - e.g. [38–51]; various substrates that stimulate H₂S production have been identified, as well as the biochemical pathways involved in H₂S production have been partially characterized. L-cysteine was found a common substrate in many bacterial species - e.g. [45,46]. In addition, thiosulfate has been shown to be a stimulator of bacterial H₂S production *via* the action of bacterial thiosulfate reductase [47].⁶ It is now clear that the mammalian enzymes responsible for H₂S production (cystathionine-beta-synthase, CBS; cystathionine-gamma-lyase, CSE and 3-mercaptopyruvate sulfurtransferase, 3-MST) have bacterial homologs with similar function.⁷ For example, in *Klebsiella pneumoniae*, cystathionine β-synthase is the main source of H₂S, and it is encoded by *mtcB* [48], while in *E. coli*, 3-MST is the main source of H₂S, and it is encoded by *mstA* [49,50].

For many decades, the actual function of the bacterially produced H₂S remained largely unclear; it was considered a source of foul smell in wastewaters, perhaps as an environmental hazard, or, at best, an indicator of bacterial overgrowth in spoiled dairy or meat products [44].

Starting with Rizzo's work in 1967, who demonstrated the production of H₂S in periodontal pockets of patients [54], a distinct line of studies focused on H₂S production by components of the oral microbiota, largely as a source of oral halitosis [45,54–58] or perhaps as a

⁵The eggy smell of eggs, then, at least in part, is due to H₂S: H₂S production by eggs has been subsequently confirmed by several groups (e.g. 33). However, whether H₂S in eggs has any biological role remains unclear. It is worth noting that in 2009, we have demonstrated (34) that H₂S stimulates angiogenesis in chicken chorioallantoic membranes (developing chick embryo), and that inhibitors of H₂S production suppress angiogenesis in this experimental system.

⁶In mammalian systems, thiosulfate has been long considered an inactive degradation product of H₂S, until multiple lines of evidence (52,53) showed that thiosulfate can, in fact, act as a substrate for mammalian H₂S biosynthesis.

⁷From the evolutionary standpoint, the correct way of stating would be to say that the bacterial enzymes have mammalian homologs.

contributor to enamel damage associated with periodontal inflammation [59]. The severity of oral halitosis (and the efficacy of antibacterial mouth rinses) is commonly quantified using the Halimeter, a device that was designed as a H₂S gas sensor for dentists [57,58].^{8,9}

Another line of research focused on the production of H₂S by bacteria of the intestinal microbiota, initially mainly as a component of flatus [62], but subsequently also as a potential regulator of intestinal epithelial cell function (proliferation, energetics) and colon function (mucus formation and inflammatory and carcinogenic responses) - as one of the many “effector” molecules produced by the intestinal microbiome) [63–67] as well as a potential source of circulating H₂S for the host [67,68].

A recent line of studies, conducted in a range of bacteria (*B. anthracis*, *P. aeruginosa*, *S. aureus*, and *E. coli*) by Nudler and colleagues (New York University School of Medicine) placed bacterial H₂S production into a new light: multiple lines of data suggest that bacterial H₂S acts as an endogenous, bacterial-derived protective factor, which renders these pathogens highly resistant to a multitude of antibiotics [49,50]. Similarly, bacterially produced H₂S confers resistance to elimination by the immune system *in vitro* and *in vivo* [69]. These studies then identify the bacterial H₂S-producing enzymes as potential targets for antimicrobial intervention. Interestingly, the idea that bacterial H₂S confers bacterial resistance has a precursor study from the mid-60’s: Schutzenberger and Bennett (University of Houston, Texas), working with *S. aureus*/*E. coli* co-cultures noticed that the presence of *E. coli* (which was the more prominent H₂S producer of the two bacteria) markedly reduced the sensitivity of the co-cultured *S. aureus* to mercury-based antibiotics and suggested that H₂S (as a secreted, diffusible mediator) is responsible for this effect [43].

Not only bacteria, but a variety of non-mammalian species produce H₂S, and utilize it for various biological processes. Plant-derived H₂S production was first observed by De Cormis in 1968 [70]. It is now well established various plants produce H₂S (primarily from sulfate), and it functions not only as a way to contribute to sulfur elimination, but also as a protective (e.g. fungo-toxic) and signaling molecule [71].

Marine biology has several interesting H₂S-related aspects, one of which being the description [72] by Fenchel and Riedel the “sulfide system” as a new biotic community present underneath marine sediments worldwide. The next major discovery came after the discovery by Jack Corliss, in 1977, the existence of deep-sea hydrothermal vents, and their surrounding rich biological environments. The extensive sulfur chemistry/biology in deep sea hydrothermal vents - first described in the early 80’s - represents an example of ancient, life-supporting functions of H₂S, with important evolutionary implications [73–75]. H₂S is sometimes called “the sunlight of the deep ocean”, because, in this dark environment, H₂S, emitted from the deep-sea volcanic vents is a vital source of metabolic energy for the primary producers of living matter - reviewed in [75,76]. In this environment, many sulfo-oxidant bacteria derive their metabolic energy from driving electrons from sulfide to oxygen.

⁸It should be noted, however, that this electrochemical sensor is not absolutely specific for H₂S and also produces a signal with other small volatile sulfhydryl compounds such as methanethiol.

⁹We have subsequently repurposed this device to measure, in animals and in human volunteers, exhaled H₂S levels (basal levels as a result of physiological processes, and increased levels after H₂S donor administration) [60,61].

In addition, multicellular organisms (such as tubeworms) living in these deep-sea-vent “biological oases” utilize H_2S as a primary source of energy. In this system, the worm takes up HS^- from its environment, and, in turn, delivers sulfide to its internal bacterial symbionts, which, in turn, utilize it. According to one line of evolutionary theory, billions of years ago, life evolved near deep sea vents, and was therefore based on sulfide chemistry (rather than oxygen chemistry, as, at this time oxygen did not yet exist on the planet in significant amounts) [75–79].

From the multitude of non-mammalian life forms, the H_2S -related aspects of the model organism *C. elegans* is also worth noting, because this organism is commonly used to address fundamental questions related to stress, lifespan and longevity. The first study to examine the effect of exogenously applied H_2S on the life span of *C. elegans* was published by Miller and Roth in 2007 [80]. Several of the subsequent studies have focused on endogenously produced H_2S in this organism. Similar to mammals and bacteria, three H_2S -synthesizing enzymes are present in *C. elegans*, namely CSE, CBS and 3-MST, and regulate cell signaling, signal transduction, bioenergetics and lifespan [81–86].

4. H_2S as an endogenous mammalian mediator

The history of H_2S - as a mammalian product - dates back to the work of the American biochemist Vincent Du Vigneaud (1901–1978). “*If one views the totality of du Vigneaud’s contributions to science, one recognizes a thread of continuity connecting sulfur-containing, biologically important compounds. This thread extends from insulin to cysteine, homocysteine, methionine, cystathionine, biotin, penicillin, oxytocin, and vasopressin*” [87]. His work on oxytocin in the 1950’s (which included the delineation of its sequence, and its successful chemical synthesis) was recognized with the Nobel Prize in 1955.

However, a decade prior to his work on oxytocin, in the 1930’s and 1940’s, first at Washington University, and subsequently at Cornell University, Du Vigneaud started working on the oxidation of sulfur-containing amino acids in tissues and in whole animals [88]. discovered and named the transsulfuration pathway, a metabolic pathway involving the interconversion of cysteine and homocysteine, through the intermediate cystathionine. Du Vigneaud described this pathway in various mammalian tissues, including liver homogenates and originally simply termed in “transulfuration” [88].¹⁰ To prove the importance of the transsulfuration pathway, Du Vigneaud synthesized L-cystathionine, and demonstrated that this compound sustained growth of rats on a cysteine-deficient diet [89]. It was during the course of a series of transsulfuration-related studies, in a paper published by Francis Binkley and Vincent Du Vigneaud in 1942, the production of cysteine from homocysteine and serine was measured in liver homogenates, and data were presented to show the “production of H_2S ” [90]. The method for the H_2S quantification was not disclosed in the paper, and the amount of H_2S was noted with (++ or +++ symbols only). Surprisingly - other than a side note claiming that cyanide was able to inhibit H_2S formation from the homogenates - the authors did not make any speculation with respect to any of the potential biological role of

¹⁰Originally the word was spelled with one “s”; later on, the current spelling (transsulfuration) became widely accepted. Also, mammalian transulfuration was later re-named as “reverse transulfuration”, while the bacterial transulfuration system became known as “forward transulfuration”.

the H₂S formed, even though, at the time of this paper, bacterial H₂S production was already a known phenomenon (see previous section).¹¹ After 1942, to our knowledge, Du Vigneaud no longer studied mammalian H₂S production, nor did he mention it in his later papers. We must conclude that he must have failed to recognize its potential biological significance.

After Du Vigneaud's early observations, for several decades, no published work can be found on the potential endogenous formation of H₂S in mammalian cells or tissues. In the 60's and 70's, several groups of investigators have conducted increasingly more detailed and sophisticated characterizations of the biochemical properties of the enzymes of the transsulfuration pathway, including cystathionine-beta-synthase (CBS) and cystathionine-gamma-lyase (CSE), and in several of these reports, the H₂S produced by purified enzymes or tissue extracts (commonly, rat liver homogenates) was also determined (typically quantified as nmoles of H₂S/min per mg of protein) [91–99].¹² These early reports include a study from Hylin and Wood showing that 3-MST results in polysulfide formation [99].¹³ More detailed reports, published in the early 80's, came from Stipanuk's group, who have demonstrated that CBS and CSE (from L-cysteine and homocysteine) [101] as well as 3-MST (from 3-mercaptopyruvate) [102] can stimulate H₂S generation in various mammalian tissues. However, these studies, as far as H₂S was concerned, remained at the level of biochemical description, and there were no suggestions (much less experimental studies) to presume or to test any potential biological effect or relevance of this gas. In other words, until the early 90's, the mammalian production of H₂S, although already known on a biochemical level, was merely viewed as a biochemical phenomenon (of some interest), rather than a (potential) biological regulatory mechanism.

In 1989/1990, three independent groups - Warenycia and co-workers¹⁴ [103], Goodwin and co-workers [104], and Savage and Gould [105] reported their findings on the concentrations of H₂S in brain tissues of animals [103] or human subjects [104,105] exposed to toxic doses of H₂S. Although the original motivation of the work was to conduct toxicological/toxicokinetic analysis, to improve the area of environmental toxicology, in these papers, the surprising finding was also noted that normal brain tissues - i.e. in the absence of any external H₂S exposure - contain significant amounts of H₂S. For instance, Warenycia and co-workers quantified basal brain H₂S levels as 1.2–1.7 µg/g tissue [103].¹⁵ In this report, the first speculation appears with respect to biological production and its potential physiological or pathophysiological role.¹⁶

¹¹It is remarkable that in a 50-page comprehensive biography prepared by Klaus Hofmann for the National Academy of Sciences published in 1987 - although the concept of transsulfuration is discussed in detail - there is no mention of Du Vigneaud's contribution to the biological production of H₂S. Nor is the pioneering work of Du Vigneaud mentioned in any of the last decades' review articles focusing on pathways of endogenous H₂S production.

¹²Many of these early reports can only be found in the early German and French scientific literature.

¹³Subsequent work, by Kimura's group demonstrated that polysulfides, on their own (i.e. without conversion to H₂S) act as biological mediators involved, among others, in the post-translational modification of proteins via sulphydration [100].

¹⁴Not surprisingly, a lot of the toxicological work on H₂S comes from Alberta, Canada, where H₂S exposure in the petrochemical industry has been a serious problem. Warenycia and co-workers were based at the University of Edmonton, and this particular, pioneering piece of work was supported by Alberta Community and Occupational Health through the Heritage Trust Research Program.

¹⁵The absolute concentrations of biologically produced H₂S have been and remain controversial; it is now widely accepted that in these early reports, the absolute values of H₂S production have been overestimated due to methodological issues.

¹⁶Although the exact function of this sulfide is unknown, there are enzymatic processes that lead to the formation of H₂S and to its rapid metabolism *in vivo*. Therefore, it remains to be seen whether endogenous levels are of physiologic significance with respect to

Inspired by the above reports, Abe and Kimura (at the time, working at the Salk Institute in San Diego) decided to conduct direct experiments to test the potential role of H₂S as an endogenous mediator in the CNS. The results of the resulting paper, published in 1996 [106] were two-fold. First, the study showed that CBS is highly expressed in the brain, it produces H₂S and this can be blocked by prototypical CBS inhibitors (aminooxyacetate, hydroxylamine). Second, it showed that low concentrations of H₂S (concentrations, that were considered physiological by the authors of the study at the time) affect neuronal function (e.g. enhance NMDA-mediated responses and facilitate long-term potentiation). Although the studies outlined in the paper did not definitely prove the functional role of *endogenously produced H₂S* in the brain (e.g. it did not include studies examining the effect of inhibition of endogenous H₂S production on neuronal functions), based on the data, Kimura suggested that endogenous H₂S plays a functional role in the regulation of neuronal function. The paper became one of the highest-cited original paper in the field of H₂S biology (receiving over 1,500 citations up till now) and it is widely considered a starting point for follow-up studies testing the functional role of endogenously produced H₂S in various mammalian systems. After the paper was finally published in the *Journal of Neuroscience* in 1996, the preeminent neuroscientist/pharmacologist Solomon Snyder wrote an enthusiastic commentary in *Science News* [107], in which he stated: “*They have very impressive evidence that H₂S is a potential neurotransmitter. It’s an exciting paper that should stimulate a lot of people’s interest.*”¹⁷

However, it took some time until this interest became apparent. Nevertheless, Kimura and his group continued his pioneering efforts in the field. In a separate line of studies, published in 1997, the Kimura group focused on smooth muscle function, and demonstrated the presence of H₂S-producing enzymes in vascular tissue, and showed the smooth muscle relaxant effect of H₂S, alone and in synergy with nitric oxide [108]. Once again, the functional effect of inhibition of endogenously produced H₂S was not studied; nevertheless, the totality of the data suggested the emergence of a new endogenous regulatory factor (gasotransmitter) in the neuronal and cardiovascular system.

Rui Wang (at the time: University of Saskatchewan, Saskatoon, Canada) became interested in further investigating the cardiovascular regulatory roles of H₂S. In his 2001 paper in the *EMBO Journal* [109], he set out to address the following questions: “*Does H₂S affect the cardiovascular function in vivo of whole animal or in vitro at the cellular level? Do the cardio-vascular effects of H₂S have physiological significance?*” In a series of elegant studies, his group has found answers to some of these questions, and demonstrated, among others, the importance of K_{ATP} channels in the vascular relaxant effect of H₂S, and proved the role of endogenous H₂S in the regulation of vascular tone and blood pressure [109–114]. He also proposed that H₂S is the “third” biological gas (or “*gasotransmitter*”); the first and second ones being nitric oxide (NO) and carbon monoxide (CO), respectively [115–117]. Thus, although it might have taken several centuries, H₂S was promoted from a toxic

mechanisms underlying the control of neuronal excitability. Furthermore, it will be of interest to determine whether elevation of intraneuronal or CNS sulfide levels correlations with known pathophysiological states...” [63].

¹⁷One of these people was Professor Snyder himself. Although it took more than 10 years after Abe and Kimura’s paper until Snyder’s first paper on H₂S appeared in the literature, his group has contributed significantly to the field, among others by identifying the process of sulphydration (posttranslational modification of proteins) (see below for further details).

substance and environmental hazard into an important endogenous mammalian biological messenger. Some of the key observations along the way are reiterated in Table. 1.

Over the last decade, a large number of groups have entered the field of H₂S biology, and the field exploded in terms of quality and quantity of data (Fig. 1, Tables 2–4) [118–153]. Table 2 lists the most highly cited publications in the field of H₂S biology, primarily over the last decade. Many of these highly cited papers can be grouped into several (in part, overlapping) categories: neuroscience [106,119,132,135,140,148], cardiovascular [34,107–113,114,118,121,123,127,128,130,133,136,139,141,145,152,153], inflammatory/cell death signaling [122,124–126,138,142,143] and metabolism [28,85,141,147,150]. Moreover - following the early observation of the formation of a nitrosothiol from the reaction of NO and H₂S [154] - additional studies have investigated the various chemical and biological interactions between the NO and the H₂S pathway [145,146,152,153,155]. The current “output” is approximately 1,000 published publications per year (according to PubMed) (Fig. 1). For the last decade, the exchange of information and the collaborations in the field of H₂S biology have been significantly fostered by regular scientific meetings focusing on the biology and medicine of H₂S (Table 3).

From the highly cited reports highlighted in Table 2, as well as thousands of additional studies published over the last decade, it became increasingly clear that H₂S, at low concentrations (or low, steady rates of generation, in case of H₂S donor compounds), has an entirely different pharmacological profile than what was previously characterized in the toxicological literature (where, typically, high concentrations were used to demonstrate adverse/noxious effects). Hence, the often-mentioned concept of the “bell-shaped pharmacological profile of H₂S” was born. For example, at low concentrations, H₂S was found to be cytoprotective [118,119,124,127,152] (as opposed to the cytotoxicity observed at higher concentrations). In addition, H₂S turned out to have antioxidant/free radical and oxidant-scavenging effects [119,120,140], in addition to the previously known pro-oxidant and cytotoxic effects. H₂S, at low concentrations, was also recently recognized to exert protective effects against DNA damage [156,157] - as opposed to its previously established DNA-damaging actions seen at high concentrations. Various anti-inflammatory effects of H₂S have also been demonstrated [122], in stark contrast to the previous concept that proclaimed that H₂S promotes inflammation. Finally, H₂S, when exogenously administered at low concentrations, or when endogenously produced at biological rates, was found to exert stimulatory effects on cellular respiration [144,147,150,158,159] - in stark contrast to the well-established inhibitory effect on Complex IV, which only became apparent at higher concentrations.

With the use of pharmacological inhibitors, as well as mice lacking various H₂S-producing enzymes, and by using transient and stable silencing of H₂S-producing enzymes, the functional role of endogenously produced H₂S is being delineated in more detail (as opposed to testing the effect of low concentrations of exogenously applied H₂S on biological systems). There were also significant advances made in the field of H₂S-mediated signaling, including the concept of sulfhydration (post-translational modification of protein cysteine residues, with functional consequences), which is prominently induced by polysulfides [114,138,142,143]. Indeed, polysulfides are emerging as a distinct class of sulfur-containing

biological signaling molecules, with properties that are different from those of H₂S [148]. A separate pathway of H₂S production, from D-cysteine, has also been discovered by Kimura and his group in 2013, with relevance for the kidney and possibly other organs, as well [151]. Another area within the growing field of H₂S biology relates to the interactions of H₂S with various oxygen- and nitrogen-derived species, which includes functional interactions (for example at the level of the Akt signaling and the cGMP signaling) [145,152] as well as the recognition of the biological roles of various hybrid S/N species [146,155].¹⁸

The above mechanistic revelations (as well as many additional lines of studies that are too numerous to be mentioned here in detail) have also led to new concepts focusing on pharmacological supplementation (donation) of H₂S, for therapeutic benefit, for pathophysiological states where H₂S levels are diminished (either due to decreased production or increased consumption), including cardiovascular disease states (e.g. ischemia-reperfusion, vascular disease) [118,127,133] and to induce cytoprotection against the toxic side effects of various drugs [124]. These effects, at least in part, may also be related to the newly recognized proangiogenic effects [34,128] of H₂S. It may be worth mentioning that the roots of the cardiovascular protective effect of H₂S can be traced back to a parallel line of studies that began in the 50's, focusing on balneotherapy (beneficial effects of H₂S-containing spring baths) [162–164].¹⁹ The first long-acting H₂S donor, synthesized and characterized by Philip Moore's group in Singapore, was designated as GYY4137; this compound was found to exert beneficial blood pressure and vascular effects in the initial report [130], and was subsequently used in many dozens of follow-up studies and showed therapeutic benefit in many systems. The synthesis of GYY4137 was followed by the synthesis and characterization of multiple classes of H₂S donors, some of them with targeting to various organelles (e.g. mitochondria) [156,165]. Another interesting group of H₂S donors is the polysulfides, which include garlic-derived natural compounds, with beneficial vascular effects [129]. Several groups have also begun synthesizing H₂S donor compounds where a known drug (e.g. a non-steroidal anti-inflammatory) was coupled with a H₂S donor group; the most advanced member of this type of compounds have progressed into Phase II clinical trials [166].

In contrast to cardiovascular diseases, where H₂S levels are decreased, it was also recognized that in many other disease conditions - e.g. various forms of systemic inflammation [122], as well as several forms of cancer, including colon cancer [150] - circulating H₂S levels are *increased* due to the upregulation of H₂S-producing enzymes; in

¹⁸(The roots of the chemical interactions between H₂S and nitrogen-containing reactive species can be traced back to the German chemistry literature [160,161]; however, the recognition that such species have biological relevance is a recent one.

¹⁹Much of this information can be found in the Russian-language literature. For instance, in the study by Kubli, rabbits were fed with cholesterol during 25–30 days (to induce hypercholesterolemia) and for 120–140 days (to induce atherosclerosis). H₂S baths with H₂S concentrations of 150–180 mg/kg were applied in the course of 12–15 procedures, every other day, at 37 °C, for 15 min. Control group received regular water bath. Activity of cytochrome oxidase was measured in heart, liver, kidney, adrenal glands and hypothalamus by colorimetric assay. The study found that experimental atherosclerosis lead to decreased activity of cytochrome oxidase in all studied organs even at early stage of pathology (25–30 days); H₂S bath increased activity of cytochrome oxidase, particular in heart and hypothalamus. In another study by N.R. Chepikova, atherosclerosis was induced in by cholesterol feeding and via decreasing thyroid function by 6-methylthiouracil for 170 days. H₂S bath therapy was administered similar to the rabbit study outlined above. H₂S baths improved hemodynamic state in atherosclerosis dogs; restored arterial blood pressure and improved vascular function.

these conditions, pharmacological inhibition of H₂S biosynthesis shows therapeutic potential.

The current article cannot cover all aspects of the explosion in the field that occurred over the last decade. The state-of-the-art of H₂S biology can be overviewed in general reviews and monographs [116,167–174] as well as in specialized review articles focusing on the roles of H₂S in the regulation of nervous system [175–179], cardiovascular system [180–186], gastrointestinal system [187–190], renal system [191–193], metabolic and mitochondrial aspects [66, 194–196], cellular signaling [197–202], and the biochemistry of the various H₂S-producing enzymes [203–205]. Separate articles focus on the details of the chemical properties and reactivity of H₂S and its functional interactions with other reactive species including oxygen-derived oxidants and free radicals and NO [206–214]. Other review articles focus on pharmacological donors and inhibitors of H₂S biosynthesis [215–218], and on the therapeutic aspects and translational potential of H₂S biology [218–225].

Although the field of H₂S biology has dramatically expanded over the last decade, many topics and issues remain that will demand continuing attention. Some of these “hot” issues are listed in Table 4 by investigators currently active in the field. It is hoped that the current article not only gives a somewhat accurate overview of the field, but also stimulates future work in this area.

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Abbreviations

ATP	adenosine triphosphate
CBS	cystathionine-β-synthase
CSE	cystathionine gamma lyase
H₂S	hydrogen sulfide
3-MST	3-mercaptopyruvate sulfurtransferase
NO	nitric oxide
ROS	reactive oxygen species

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Figure 1. Number of published articles on H₂S per year

The figures are based on PubMed literature search and do not distinguish between biology or physics or chemistry related papers. The number of 2017 publications is a projection assuming that the publication rate seen in the first 8 months of the year continues at the same rate.

Table 1
Chronology of hydrogen sulfide research

See the text for further context and literature citations.

1700	Recognition of the adverse health effects of “sewer gas”
1775–1776	Discovery and elucidation of the chemical composition of H ₂ S
1785	Recognizing the role of H ₂ S in the intoxication of sewer workers in Paris
1803–present	Animal experiments start: testing the toxicity of H ₂ S (inhaled or injected, high doses)
1862	Literary description of sewer workers’ diseases (V. Hugo - Les Miserables)
1863	Sulfhemoglobin formation in response to H ₂ S
1865	Suggestion that H ₂ S induces “oxygen hunger” (chemical hypoxia)
1877–1884	Early experiments demonstrating that bacteria produce H ₂ S
1917–1960’s	H ₂ S production by multiple bacterial species, role of cysteine, partial characterization of the enzymes involved
1940	Demonstration that H ₂ S inhibits cytochrome C oxidase activity
1942	Demonstration of H ₂ S production in mammalian tissues (during transsulfuration reactions)
1950–1970’s	H ₂ S production in mammalian cells and tissues, role of cysteine, partial characterization of the enzymes involved
1948	Evidence for the chemical formation of hybrid S/N species
1959	Enzymatic formation of polysulfides from 3-mercaptopyruvate
1965	Bacterial co-culture experiments suggesting the role of bacterial H ₂ S as a factor that confers resistance against antimicrobials
1966–1970	Protective effects of H ₂ S administration (balneotherapy) on cardiac and vascular function in animals
1967	Suggestion that H ₂ S, produced by oral bacteria, contributes to halitosis
1968	Demonstration of H ₂ S production in plants
1970	Description of a widespread H ₂ S system in marine sediments
1975	Purification and biochemical characterization of CBS
1977	Discovery of deep-sea hydrothermal vents and associated biological communities
1980–present	Characterization of H ₂ S-based biological environments in deep-sea hydrothermal vents
1982	Detailed biochemical characterization of CBS and CSE-mediated H ₂ S formation
1983	Detailed biochemical characterization of 3-MST-mediated H ₂ S formation
1989–90	Evidence of detectable levels of (endogenous) H ₂ S in brain samples from animals and humans
1986	H ₂ S oxidation is coupled to oxidative phosphorylation in mitochondria of <i>Solemya reidi</i>
1996	Proof of H ₂ S-producing enzymes in the brain; effects of low concentrations of H ₂ S on neuronal function
1997	Proof of H ₂ S-producing enzymes in blood vessels; effects of low concentrations of H ₂ S on vessel function (in synergy with NO)
1998	Suggestions linking enteric H ₂ S production to colonic inflammation
2001	Implication of K _{ATP} channels in the vascular effects of H ₂ S
2003	Endogenous H ₂ S is overproduced in Down Syndrome
2004	Demonstration of free radical/oxidant scavenging effects of H ₂ S
2004–present	Antioxidant/cytoprotective effects of H ₂ S
2005	Demonstration of H ₂ S-induced ‘hibernation’ in mice

2005	Demonstration of the pathogenic role of H ₂ S overproduction in sepsis
2005	Inhibition of hydrogen sulfide generation contributes to gastric injury caused by anti-inflammatory nonsteroidal drugs
2006	Demonstration of DNA damage induced by H ₂ S (high concentrations)
2006	Hydrogen sulfide suppresses leukocyte-mediated inflammation
2006	Formation of a nitrosothiol from the reaction of NO and H ₂ S
2007	Demonstration of the effect of H ₂ S on lifespan in C Elegans
2007	H ₂ S generation from garlic-derived polysulfides
2007	Proangiogenic effect of H ₂ S donation
2007-present	Beneficial effect of H ₂ S donation in cardiovascular, renal and inflammatory diseases and cancer
2007-present	Multiple lines of studies investigating the mode of H ₂ S's cytoprotective, anti-inflammatory and preconditioning effects
2007	Cardioprotective effect of H ₂ S in coronary ischemia-reperfusion
2007	Sulfide as an inorganic substrate of the mitochondrial electron transport in human cells
2008	GY4137: synthesis and characterization of a slow-release H ₂ S donor
2008-present	Synthesis and characterization of multiple classes of H ₂ S donors and combined donors
2009	Proangiogenic effect of endogenous H ₂ S
2009	Sulfhydration: a posttranslational process triggered by H ₂ S
2010	Human clinical trials with IK-1001 (sodium sulfide for i.v. injection)
2011	Proving the role of bacterial H ₂ S as a factor that confers resistance against antimicrobials
2013	Polysulfides as signaling molecules in the CNS
2013	Demonstration of the pathogenic role of H ₂ S overproduction in cancer and therapeutic potential of CBS inhibition
2014	First reports on the synthesis and characterization of mitochondrially targeted H ₂ S donors
2014–2016	Demonstration that H ₂ S facilitates DNA repair
2015	Demonstration that H ₂ S production is required for dietary restriction benefits
2016	Phase II clinical trials with ATB-346, a H ₂ S-releasing nonsteroidal anti-inflammatory drug development candidate

Table 2
Highly cited papers in the field of hydrogen sulfide biology

Original research articles with at least 30 citations per year (based on Google Scholar) are compiled; review articles and “methods” papers were excluded from the analysis.

Authors	Title	Reference	Citations	Reference
1996				
Abe K, Kimura H.	The possible role of H ₂ S as an endogenous neuromodulator.	J Neurosci. 16:1066–71, 1996.	1496 (71/yr)	106
1997				
Hosoki R, Matsuki N, Kimura H.	The possible role of H ₂ S as an endogenous smooth muscle relaxant in synergy with nitric oxide.	Biochem Biophys Res Commun. 237:527–31, 1997.	1091 (55/yr)	107
2001				
Zhao W, Zhang J, Lu Y, Wang R.	The vasorelaxant effect of H ₂ S as a novel endogenous gaseous K _{ATP} channel opener.	EMBO J. 2001 Nov 1;20(21):6008–16	1726 (108/yr)	109
2002				
Zhao W, Wang R.	H ₂ S-induced vasorelaxation and underlying cellular and molecular mechanisms.	Am J Physiol Heart Circ Physiol. 283:H474–80, 2002.	543 (36/yr)	110
2004				
Geng B, Chang L, Pan C, Qi Y, Zhao J, Pang Y, Du J, Tang C.	Endogenous H ₂ S regulation of myocardial injury induced by isoproterenol.	Biochem Biophys Res Commun. 318:756–63, 2004.	462 (36/yr)	118
Kimura Y, Kimura H.	H ₂ S protects neurons from oxidative stress.	FASEB J. 18:1165–7, 2004.	726 (55/yr)	119
Whiteman M, Armstrong JS, Chu SH, Jia-Ling S, Wong BS, Cheung NS, Halliwell B, Moore PK.	The novel neuromodulator H ₂ S: an endogenous peroxynitrite ‘scavenger’?	J Neurochem. 90:765–8, 2004.	536 (41/yr)	120
Cheng Y, Ndisang JF, Tang G, Cao K, Wang R.	H ₂ S-induced relaxation of resistance mesenteric artery beds of rats.	Am J Physiol Heart Circ Physiol. 287:H2316–23, 2004.	438 (34/yr)	121
2005				
Blackstone E, Morrison M, Roth MB.	H ₂ S induces a suspended animation-like state in mice.	Science. 308:518, 2005.	657 (55/yr)	28
Li L, Bhatia M, Zhu YZ, Zhu YC, Ramnath RD, Wang ZJ, Anuar FB, Whiteman M, Salto-Tellez M, Moore PK.	H ₂ S is a novel mediator of lipopolysaccharide-induced inflammation in the mouse.	FASEB J. 19:1196–8, 2005.	636 (53/yr)	122
Fiorucci S, Antonelli E, Mencarelli A, Orlandi S, Renga B, Rizzo G, Distrutti E, Shah V, Morelli A.	The third gas: H ₂ S regulates perfusion pressure in both the isolated and perfused normal rat liver and in cirrhosis.	Hepatology. 42:539–48, 2005.	368 (31/yr)	123
Fiorucci S, Antonelli E, Distrutti E, Rizzo G, Mencarelli A, Orlandi S, Zanardo R, Renga B, Di Sante M, Morelli A, Cirino G, Wallace JL.	Inhibition of H ₂ S generation contributes to gastric injury caused by anti-inflammatory nonsteroidal drugs.	Gastroenterology. 129:1210–24, 2005.	382 (32/yr)	124

Authors	Title	Reference	Citations	Reference
2006				
Oh GS, Pae HO, Lee BS, Kim BN, Kim JM, Kim HR, Jeon SB, Jeon WK, Chae HJ, Chung HT.	H ₂ S inhibits nitric oxide production and nuclear factor-kappaB via heme oxygenase-1 expression in RAW264.7 macrophages stimulated with lipopolysaccharide.	Free Radic Biol Med. 41:106–19, 2006.	411 (35/yr)	125
Zanardo RC, Brancalone V, Distrutti E, Fiorucci S, Cirino G, Wallace JL.	H ₂ S is an endogenous modulator of leukocyte-mediated inflammation.	FASEB J. 20:2118–20, 2006.	645 (58/yr)	126
2007				
Elrod JW, Calvert JW, Morrison J, Doeller JE, Kraus DW, Tao L, Jiao X, Scalia R, Kiss L, Szabo C, Kimura H, Chow CW, Lefer DJ.	H ₂ S attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function.	Proc Natl Acad Sci USA. 104:15560–5, 2007.	777 (78/yr)	127
Cai WJ, Wang MJ, Moore PK, Jin HM, Yao T, Zhu YC.	The novel proangiogenic effect of H ₂ S is dependent on Akt phosphorylation.	Cardiovasc Res. 76:29–40, 2007.	302 (30/yr)	128
Benavides GA, Squadrito GL, Mills RW, Patel HD, Isbell TS, Patel RP, Darley-Usmar VM, Doeller JE, Kraus DW.	H ₂ S mediates the vasoactivity of garlic.	Proc Natl Acad Sci USA. 104:17977–82, 2007.	508 (51/yr)	129
2008				
Li L, Whiteman M, Guan YY, Neo KL, Cheng Y, Lee SW, Zhao Y, Baskar R, Tan CH, Moore PK.	Characterization of a novel, water-soluble H ₂ S -releasing molecule (GYY4137): new insights into the biology of hydrogen sulfide.	Circulation. 117:2351–60, 2008.	404 (45/yr)	130
Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, Wang R.	H ₂ S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase.	Science. 322:587–90, 2008.	1402 (155/yr)	113
Furne J, Saeed A, Levitt MD.	Whole tissue H ₂ S concentrations are orders of magnitude lower than presently accepted values.	Am J Physiol Regul Integr Comp Physiol. 295:R1479–85, 2008.	417 (46/yr)	131
2009				
Ishigami M, Hiraki K, Umemura K, Ogasawara Y, Ishii K, Kimura H.	A source of H ₂ S and a mechanism of its release in the brain.	Antioxid Redox Signal. 11:205–14, 2009.	322 (40/yr)	132
Wang Y, Zhao X, Jin H, Wei H, Li W, Bu D, Tang X, Ren Y, Tang C, Du J.	Role of H ₂ S in the development of atherosclerotic lesions in apolipoprotein E knockout mice.	Arterioscler Thromb Vasc Biol. 29:173–9, 2009.	240 (30/yr)	133
Chiku T, Padovani D, Zhu W, Singh S, Vitvitsky V, Banerjee R.	H ₂ S biogenesis by human cystathionine gamma-lyase leads to the novel sulfur metabolites lanthionine and homolanthionine and is responsive to the grade of hyperhomocysteinemia.	J Biol Chem. 284: 11601–12, 2009.	244 (31/yr)	134
Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, Ishii K, Kimura H.	3-Mercaptopyruvate sulfurtransferase produces H ₂ S and bound sulfane sulfur in the brain.	Antioxid Redox Signal. 11:703–14, 2009.	550 (69/yr)	135
Calvert JW, Jha S, Gundewar S, Elrod JW,	H ₂ S mediates cardioprotection through Nrf2 signaling.	Circ Res. 105:365–74, 2009.	424 (53/yr)	136

Authors	Title	Reference	Citations	Reference
Ramachandran A, Pattillo CB, Kevil CG, Lefer DJ.				
Singh S, Padovani D, Leslie RA, Chiku T, Banerjee R.	Relative contributions of cystathionine beta-synthase and gamma-cystathionase to H ₂ S biogenesis via alternative trans-sulfuration reactions.	J Biol Chem. 284:22457–66, 2009.	290 (36/yr)	137
Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK, Barrow RK, Yang G, Wang R, Snyder SH.	H ₂ S signals through protein S-sulfhydration.	Sci Signal. 2:ra72, 2009.	600 (75/yr)	138
Shibuya N, Mikami Y, Kimura Y, Nagahara N, Kimura H.	Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces H ₂ S.	J Biochem. 146:623–6, 2009.	345 (43/yr)	139
Papapetropoulos A, Pyriochou A, Altaany Z, Yang G, Marazioti A, Zhou Z, Jeschke MG, Branski LK, Herndon DN, Wang R, Szabo C.	H ₂ S is an endogenous stimulator of angiogenesis.	Proc Natl Acad Sci USA. 106:21972–7, 2009.	432 (54/yr)	34
2010				
Kimura Y, Goto Y, Kimura H.	H ₂ S increases glutathione production and suppresses oxidative stress in mitochondria.	Antioxid Redox Signal. 12:1–13, 2010.	364 (52/yr)	140
Peng YJ, Nanduri J, Raghuraman G, Souvannakitti D, Gadalla MM, Kumar GK, Snyder SH, Prabhakar NR.	H ₂ S mediates O ₂ sensing in the carotid body.	Proc Natl Acad Sci USA. 107:10719–24, 2010.	234 (33/yr)	141
2011				
Mustafa AK, Sikka G, Gazi SK, Steppan J, Jung SM, Bhunia AK, Barodka VM, Gazi FK, Barrow RK, Wang R, Amzel LM, Berkowitz DE, Snyder SH.	H ₂ S as endothelium-derived hyperpolarizing factor sulfhydrates potassium channels.	Circ Res. 109:1259–68, 2011.	310 (52/yr)	114
Shatalin K, Shatalina E, Mironov A, Nudler E.	H ₂ S: a universal defense against antibiotics in bacteria.	Science. 334:986–90, 2011.	240 (40/yr)	49
Krishnan N, Fu C, Pappin DJ, Tonks NK.	H ₂ S-induced sulfhydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response.	Sci Signal. 4:ra86, 2011.	211 (35/yr)	142
2012				
Sen N, Paul BD, Gadalla MM, Mustafa AK, Sen T, Xu R, Kim S, Snyder SH.	H ₂ S-linked sulfhydration of NF- κ B mediates its antiapoptotic actions.	Mol Cell. 45:13–24, 2012.	296 (60/yr)	143
Fu M, Zhang W, Wu L, Yang G, Li H, Wang R.	H ₂ S metabolism in mitochondria and its regulatory role in energy production.	Proc Natl Acad Sci USA. 109:2943–8, 2012.	180 (36/yr)	144
Coletta C, Papapetropoulos A, Erdelyi K, Olah G, Módis K, Panopoulos P, Asimakopoulou A, Gerö D, Sharina I, Martin E, Szabo C.	H ₂ S and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation.	Proc Natl Acad Sci USA. 109:9161–6, 2012.	278 (42/yr)	145
Filipovic MR, Miljkovic LJ, Nauser T, Royzen M, Klos K, Shubina T, Koppenol	Chemical characterization of the smallest S-nitrosothiol, HSNO;	J Am Chem Soc. 134:12016–27, 2012.	171 (34/yr)	146

Authors	Title	Reference	Citations	Reference
WH, Lippard SJ, Ivanovi-Burmazovi I.	cellular cross-talk of H ₂ S and S-nitrosothiols.			
2013				
Módis K, Coletta C, Erdélyi K, Papapetropoulos A, Szabo C.	Intramitochondrial H ₂ S production by 3-mercaptopyruvate sulfurtransferase maintains mitochondrial electron flow and supports cellular bioenergetics	FASEB J. 27: 601–611, 2013.	118 (30/yr)	147
Kimura Y, Mikami Y, Osumi K, Tsugane M, Oka J, Kimura H.	Polysulfides are possible H ₂ S-derived signaling molecules in rat brain.	FASEB J. 27:2451–7, 2013.	146 (37/yr)	148
Asimakopoulou A, Panopoulos P, Chasapis CT, Coletta C, Zhou Z, Cirino G, Giannis A, Szabo C, Spyroulias GA, Papapetropoulos A.	Selectivity of commonly used pharmacological inhibitors for cystathionine β synthase (CBS) and cystathionine γ lyase (CSE).	Br J Pharmacol. 169:922–32, 2013.	135 (34/yr)	149
Szabo C, Coletta C, Chao C, Módis K, Szczesny B, Papapetropoulos A, Hellmich MR.	Tumor-derived H ₂ S, produced by cystathionine-β-synthase, stimulates bioenergetics, cell proliferation, and angiogenesis in colon cancer.	Proc Natl Acad Sci USA. 110:12474–9, 2013.	165 (41/yr)	150
Shibuya N, Koike S, Tanaka M, Ishigami-Yuasa M, Kimura Y, Ogasawara Y, Fukui K, Nagahara N, Kimura H.	A novel pathway for the production of H ₂ S from D-cysteine in mammalian cells.	Nat Commun. 4:1366, 2013.	170 (42/yr)	151
2014				
King AL, Polhemus DJ, Bhushan S, Otsuka H, Kondo K, Nicholson CK, Bradley JM, Islam KN, Calvert JW, Tao YX, Dugas TR, Kelley EE, Elrod JW, Huang PL, Wang R, Lefer DJ.	H ₂ S cytoprotective signaling is endothelial nitric oxide synthase-nitric oxide dependent.	Proc Natl Acad Sci USA. 111:3182–7, 2014.	131 (43/yr)	152
Eberhardt M, Dux M, Namer B, Miljkovic J, Cordasic N, Will C, Kichko TI, de la Roche J, Fischer M, Suárez SA, Bikiel D, Dorsch K, Leffler A, Babes A, Lampert A, Lennerz JK, Jacobi J, Martí MA, Doctorovich F, Högestätt ED, Zygmunt PM, Ivanovic-Burmazovic I, Messlinger K, Reeh P, Filipovic MR.	H ₂ S and NO cooperatively regulate vascular tone by activating a neuroendocrine HNO-TRPA1-CGRP signalling pathway.	Nat Commun. 5:4381, 2014.	116 (38/yr)	153
2015				
Hine C, Harputlugil E, Zhang Y, Ruckenstein C, Lee BC, Brace L, Longchamp A, Treviño-Villarreal JH, Mejia P, Ozaki CK, Wang R, Gladyshev VN, Madeo F, Mair WB, Mitchell JR.	Endogenous hydrogen sulfide production is essential for dietary restriction benefits.	Cell. 160:132–44. 2015.	94 (47/yr)	85
Cortese-Krott MM, Kuhnle GG, Dyson A, Fernandez BO, Grman M, DuMond JF, Barrow MP, McLeod G, Nakagawa H, Ondrias K,	Key bioactive reaction products of the NO/H ₂ S interaction are S/N-hybrid species, polysulfides, and nitroxyl.	Proc Natl Acad Sci USA. 112: E4651–60, 2015	76 (38/yr)	155

Authors	Title	Reference	Citations	Reference
Nagy P, King SB, Saavedra JE, Keefer LK, Singer M, Kelm M, Butler AR, Feelisch M.				

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Table 3

Conferences on hydrogen sulfide biology.

Meeting	Date	Location	Main organizer(s)
1st International Conference of Hydrogen Sulfide in Biology and Medicine	June 26–28, 2009	Shanghai, China	Rui Wang & Yi Zhun Zhu
1st European Conference on the Biology on Hydrogen Sulfide	June 15–18, 2012	Smolenice, Slovakia	Karol Ondrias
2nd International Conference on Hydrogen Sulfide Biology and Medicine	September 20–22, 2012	Atlanta, GA, USA	David Lefer
2nd European Conference on the Biology of Hydrogen Sulfide	September 8–11, 2013	Exeter, UK	Matt Whiteman
3rd International Conference on Hydrogen Sulfide in Biology and Medicine	June 4–6, 2014	Tokyo, Japan	Hideo Kimura
3rd European Conference on the Biology of Hydrogen Sulfide	May 3–6, 2015	Athens, Greece	Andreas Papapetropoulos
4th International Conference on the Biology of Hydrogen Sulfide	June 3–5, 2016	Naples, Italy	Giuseppe Cirino
5th World Congress on Hydrogen Sulfide in Biology and Medicine *	June 1–3, 2018	Toronto, Canada	John Wallace

* Planned. Please note that with this meeting, the European and International conferences will be merged and are going to be held every 2 years.

Table 4
Decisive moments and future directions in hydrogen sulfide research

initial motivation to enter the field, and areas of future interest.

Investigator	Current affiliation	What and was the decisive moment when you decided to start working in the field of H ₂ S biology?	What do you consider the most important tasks to be addressed in the future of H ₂ S biology?
Giuseppe Cirino	University of Naples	In 2000/2001, we were working at the development of NSAIDS releasing NO when we came across this second mediator we thought to start a side project. The data turned out to be so interesting that the side project became the main project in a few years.	The interplay among H ₂ S, the NO pathway and eicosanoids in health and disease. To define if the H ₂ S pathway is a feasible target to develop therapeutics in major disease areas (cardiovascular, inflammation and cancer).
Martin Feelisch	University of Southampton, UK	In 1986, during my thesis work on the molecular mode of action of nitrovasodilators I "messed around" with sulfide (inorganic salts and H ₂ S gas) and many other small thiols to study soluble guanylate cyclase activation. Although I noticed that it (redox)modulated the responses of several NO-donors I didn't make much of it at the time as the focus of my work was on NO. I re-entered the field for real in 2012 by which time the NO/H ₂ S cross-talk story had surfaced across all disciplines.	How the different forms of sulfur work at a systems level, a topic that will require the analysis of metabolite fluxes under dynamic conditions.
Hideo Kimura	National Center of Neurology and Psychiatry Kodaira, Tokyo, Japan	In 1993, in the library of Salk Institute I found a metabolic map showing mammalian enzymes which can produce H ₂ S. Subsequently, I came across papers by Goodwin et al., Warenycia et al., and Savage and Gould, reporting the endogenous sulfide in mammalian brains.	Regulation is not well understood (including polysulfides).
David Lefer	Louisiana State University, New Orleans, LA, USA	In 2005, while working at Albert Einstein College of Medicine. We received a call from a group at Ikaria to discuss the potential use of H ₂ S-based therapies during heart attack. We also felt that since H ₂ S was an endogenously produced gas that it might behave similar to nitric oxide (NO) and this was of great interest to us.	To further define the precise role of each of the 3 enzymes that produce H ₂ S and map them to organs and disease states. We should also define how H ₂ S, NO, and CO interact under physiological and pathological states. We also need to develop far better H ₂ S releasing and donating drugs with long half lives and oral activity.
Philip Moore	National University of Singapore, Singapore	In 2000, I was working on NO and NOS inhibitors and looking for something 'fresh' to study. I suggested to a student doing her final year undergraduate project that we take a look at the effect of H ₂ S on the guinea pig ileum in the organ bath – 'classical' pharmacology! The results were obvious and clear cut, very interesting and in parts difficult to interpret - which turns out to be a very good description of my experience with H ₂ S over the following two decades.	Many things still need to be properly investigated. There are unresolved questions about the sensitivity and specificity of assays as well as not only how but where to measure H ₂ S in vivo? Add to this the multitude of possible targets for this gas within the cell and the picture is extraordinarily complicated. Building on the knowledge obtained to date and then translating it to develop useful therapeutics will be the challenge for the next decade.
Peter Nagy	National Institute of Oncology, Budapest, Hungary	In 2004, I often could smell the distinct odor of H ₂ S during my mechanistic studies on the redox reactions of cysteine derivatives and other biological sulfur species. This made me excited about exploring whether the rich chemical properties of the smallest thiol molecule (sulfide) could contribute to regulation of living systems. When I looked into the scientific literature I noticed that a few investigators	To further elucidate the underlying chemical mechanisms of sulfide's biological actions with special focus on the roles of reactive sulfur species regulated pathways in cancer biology. To elucidate the specific biological actions of these molecules in order to be able to most precisely target them for therapy and to avoid side effects.

Investigator	Current affiliation	What and was the decisive moment when you decided to start working in the field of H ₂ S biology?	What do you consider the most important tasks to be addressed in the future of H ₂ S biology?
		already started to research H ₂ S biology. I conducted my first experiments in 2007.	
Evgeny Nudler	New York University Langone Medical Center, New York, NY, USA	Around 2009 we've published several papers on the protective role of NO in bacteria. I was thinking that, by analogy with mammals, H ₂ S should be an important signaling molecule in bacteria as well. When we looked at hundreds of sequenced bacterial genomes we realized that virtually all of them have paralogs of mammalian 3MST or CBS/CSE. We started to examine whether H ₂ S endows bacteria with some evolutionary advantages.	With respect to bacteria it would be important to establish H ₂ S-mediated signaling pathways. To my knowledge, protein S-sulfhydration has not yet been investigated in bacteria.
Andreas Papapetropoulos	University of Athens, Greece	In 2006 I invited Drs. C. Szabo and G. Cirino to a Greek Pharmacological Society Meeting. At dinner, we discussed current projects and H ₂ S emerged as a new molecule that was potentially important in the cardiovascular system. It shared many characteristics with NO, a molecule I had been working with for 15 years. Later this year, in collaboration with Ikaria we started working on H ₂ S and angiogenesis.	Accurately measuring H ₂ S levels in cells, tissues and biological fluids, understanding how production of H ₂ S is regulated short term so that it can exert its signaling functions, how deregulation of H ₂ S levels contributes to the development of disease, discovering better pharmacological tools to modulate its levels.
Csaba Szabo	University of Texas, Galveston, TX, USA	2002. Through my interest in vascular biology, I became aware of the studies of Dr. Kimura and Dr. Wang in the early 2000's. In 2006, I started became Chief Scientific Officer of the company Ikaria and led research on various aspects of H ₂ S biology and started developing an injectable H ₂ S formulation IK-1001 and progressed it into human trials.	To translate the many basic observations in the field of H ₂ S into novel therapeutic agents. In general: to discover and develop H ₂ S biosynthesis inhibitors and H ₂ S donors and to match them with the appropriate diseases (development indications). In specific: to introduce CBS inhibitors into clinical trials for cancer.
John Wallace	University of Calgary and Antibe Therapeutics Inc., Canada	Early 2003. Myself and Giuseppe Cirino had a discussion about the possibility that H ₂ S may be a more 'benign' molecule than NO with respect to being protective in the GI tract. We began to explore the development of H ₂ S-releasing NSAIDs.	Development of simpler but reliable methods for measuring H ₂ S production (and localization) in vivo.
Matt Whiteman	University of Exeter Medical School, UK	2000. I'd followed some of the early work as a side-interest but it wasn't until Phil Moore gave a talk on H ₂ S/NO cross-talk at the National University of Singapore in 2003 where I was working at the time that I became 'properly' interested. Since then H ₂ S has been shown to be critical for mitochondrial function and I started working on developing novel slow-release mitochondria-targeted H ₂ S delivery molecules for therapeutic and agricultural use. The side-interest has now become an obsession.	There is a very long list. Primarily for me (i) following through successful studies in animal models to establishing the clinical efficacy of mitochondria-targeted H ₂ S donors and (ii) determining how much of the published effects of H ₂ S and / or its intermediates are real and not just test-tube phenomena.