Hydrogen sulfide signaling: interactions with nitric oxide and reactive oxygen species

John T. Hancock[a] and Matthew Whiteman[b]

a. Faculty of Health and Applied Sciences, University of the West of England, Bristol, England
b. University of Exeter Medical School, University of Exeter, Exeter, England

Corresponding author:
John T. Hancock
Faculty of Health and Applied Sciences
University of the West of England
Coldharbour Lane
Bristol, BS16 1QY, UK
Email: john.hancock@uwe.ac.uk

Short title: H_2S interacts with other signaling pathways

Keywords: hydrogen sulfide; nitric oxide; reactive oxygen species; redox signaling; signal transduction.

Abstract:

Signaling in cells which involves reactive compounds is well established. Reactive oxygen species and nitric oxide are known to be extremely influential in the control of a range of physiological responses in many organisms, from animals to plants. Often their generation is triggered in reaction to stress and it is common for ROS and NO metabolism to interact to give a co-ordinated response. Recently, hydrogen sulfide (H_2S) has also been found to be an important signaling molecule, being shown to be involved in vascular tone in animals. Of relevance to respiration, in plants H_2S has been shown to affect stomatal apertures and the transpiration stream while in animals H_2S has been shown to be a source of electrons for ATP synthesis in mitochondria. However, in signaling H_2S is not working in isolation and it is likely that it will have interactions with both ROS and NO. This may be at a variety of levels, from influencing the generation of such molecules, interacting directly, or competing for control of downstream signaling events. A full understanding of the impact of this toxic molecule in the control of cells requires all these factors to be taken into account.

Introduction

Small reactive compounds such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been studied as components of cell signaling pathways for nearly thirty years[1]. Such compounds include hydrogen peroxide, singlet oxygen (O_2^1) and superoxide anions (all considered as ROS), and nitric oxide (NO) and *S*-nitrosoglutathione (GSNO) which fall under the umbrella of being RNS[2-4].

There has been a long history of research on reactive species, such as ROS and RNS, with work being carried out in the past having an impact on varying aspects of biological function, in both animals and plants.. However, the signalling roles of such compounds were highlighted by the work on NO. It was known that endothelial cells released a compound, named endothelial derived relaxing factor (EDFR), that caused smooth muscle cells to relax, and this was found to be NO[1]. Of pertinence to respiratory science, in plants both NO and ROS are known to have profound effects on stomata and so to alter the transpiration stream of the plant. On the flip side of this is the generation of such compounds as ROS by the mitochondria, meaning that respiratory activity can lead to increases in the presence of reactive species[5-6].

The focus of this review is on the signaling roles of such chemicals and how they may lead to co-ordinated responses in cells. In this context, it has been known that the presence of chemicals such as ROS and RNS not only has specific effects, leading to defined responses, but that they also influence the signaling mediated by other such compounds. As many of these compounds are relatively reactive, this could be a direct effect. An example of this would be the reaction of superoxide and NO to generate peroxynitrite

($ONOO^-$) which itself can act as a cellular signal[7]. Therefore, the interplay between these compounds suggests that they might modulate the generation or removal of each other. For example, NO may influence the levels of antioxidants in cells and therefore alter the accumulation of ROS, and any resulting signaling. Certainly interactions have been reported, with H_2O_2 and NO being shown to act together in signaling pathways[8], or interaction to influence the final outcome, as seen with plant stigmas[9]. What is clear from such examples is the fact that reactive compounds such as ROS and NO would not be acting in isolation, and interactions with other reactive compounds would need to be considered.

More recently, another reactive compound has been a focus of attention, that is, hydrogen sulfide (H_2S). This molecule has been suggested to be the third gasotransmitter, along with NO and carbon monoxide[10-12]. Many recent reviews have extolled the role of H_2S as an important signal[13-18].

For a molecule, such as H_2S, to be considered as a good candidate for being a cellular signal there are certain guidelines or criteria that need to be met[19]. There would need to be a means of generation of the compound, specifically enabling its production at the right place and at the right time. This would often require the presence of a dedicated enzyme which is able to make the signal. Once the molecule has fulfilled its signaling role, normally it would need to be removed. This would be to stop further signalling, which could be detrimental, and again an enzyme would often be involved here. Once made, a signaling compound needs to get the message moved to the next relevant component in a signal transduction pathway, and often this may mean that the compound carrying the message moves, perhaps diffusing

across the cell, or even between cells. Once the compound has arrived at its destination it would need to be recognised for what it is, and therefore be able to relay a unique message, and to elicit a unique response. However, what is often missing from such a list of criteria is the ability to interact with other signaling components and pathways. Here, we suggest that H_2S has a major influence on signaling with involves ROS and RNS, and that this is one of the main roles for H_2S in cells.

H_2S is a toxic molecule

One of the main reasons for scepticism with regards the role of H_2S in signaling is the fact that it is very toxic. However, the role of H_2S in mitochondria is two fold. Firstly, at high concentrations H_2S is acting as a toxic molecule as it is a potent inhibitor of Complex IV[20] and therefore H_2S will stop ATP production with dire effects on the cell, and indeed the organism. Secondly, at lower concentrations H_2S can feed the electron transport chain electrons and so enhance ATP production[21-22] (Figure 1). This, in fact, has also the side effect of reducing the amount of H_2S in the cell, so this may be a mechanism by which cells can remove low concentrations of H_2S.

It is important to consider the toxicity of H_2S for two reasons. Firstly, cells are able to produce H_2S endogeously, and so slow levels of the activity of the enzymes involved will lead to low concentration of H_2S inside cells most of the time (this is discussed further below). This may especially be true for plant cells which use H_2S as a substrate in sulphate metabolism[23]. Secondly, organisms can be exposed to H_2S from the environment. Natural sources of H_2S include volcanoes, thermal vents[24], or from bacterial metabolism[25]. H_2S

from such sources may also be of interest because it may give a hint to the use of H_2S, or tolerance to H_2S, during the early stages of evolution, as many organisms would have developed with H_2S being present. Organisms have had to develop ways to deal with H_2S and then may have adopted H_2S for more positive uses, such as signaling. A similar argument has been put forward for the use of ROS as signals following the generation of oxygen into the atmosphere[26]. The toxicity of H_2S has also been harnessed for warfare, highlighting its devastating effects[27] but more recently the toxicity of H_2S has been brought to the fore by events on a Japanese volcano[28].

However, the toxicity of H_2S should not stop it from being used as a cellular signal. ROS were originally studied because of their toxic effects in the fight against pathogens, with neutrophils and other phagocytes being an excellent source of ROS[29]. In a similar way, RNS too have been found to have anti-pathogen effects[30]. Both ROS and RNS are now well accepted as signals and therefore adopting H_2S as a signaling component does not seem an illogical step.

Despite the fact the H_2S is evidently toxic, it can also have positive effects in the cell, which lead it to be dubbed as a gasotransmitter and a signaling agent[10-18]. In *Caenorhabditis elegans* a low concentration of H_2S bestows thermatolerance on the nematodes and even makes them live longer[31], showing that this is an effect of H_2S which appears to be totally opposite to toxicity. Of relevance for respiratory science the beneficial effects of H_2S on breathing in fish has recently been reported[32], again showing that low concentrations of H_2S have a positive effect. Therefore, it needs to be

understood how such effects are brought about, and this may well be through an influence on signaling pathways.

Evidence that H_2S can act as a signal.

It was argued above that for H_2S to be a signal dedicated enzymes are needed to generate it. In animals, enzymes such as cystathione γ-lyase (CSE), cystathione β-synthase (CBS) and 3-mercaptopyruvate sulfurtansferase (3-MST) have been shown to have the capacity to make H_2S[33], while in plants the role seems to be taken by desulfhydrases[34]. In plants too, H_2S is part of the metabolism of sulfate, so low levels will be present and able to be generated[23].

Enzymes have been found to remove H_2S too. In plants enzymes such as O-acetylserine (thiol) lyase take on this role[35-36], but as discussed above, H_2S can be metabolised and so removed by mitochondria too[21-22].

With enzymes which can make and remove H_2S the players are in place to allow this gas to be used in signaling. However, it has to be seen to have an effect, to elicit a response. Being a small hydrophobic molecule means that it is able to diffuse relatively easily in cells, and indeed across membranes and therefore across to neighbouring cells. However, there is a caveat here, in that H_2S is reactive and has the potential to react with bio-molecules on its journey. Such reactivity has recently been reviewed[37]. For example, H_2S is known to react with proteins,[38] as will be discussed further below.

Although most research on H_2S has been carried out on animals, a wide variety of processes have been found to be influenced by H_2S in both

animals and plants,. In animals it, as with NO, has been found to have profound effects in vasodilation[39]. As well as in normal physiology H_2S has been found to be involved in, or to partially mediate, a range of diseases in animals. This includes diabetes[40], atherosclerosis[41], and vascular inflammation[42]. Treatment with H_2S, or the presence of increased H_2S, has been found to be able to lessen many disease states in animals, suggesting that it has a profound role in many systems[43-46].

In plants effects of H_2S are seen too. This ranges from alteration of germination rates[47-48], changes in stomatal apertures[49-55], root development[56-57], flower senescence[58], and the idea that H_2S can be administered to crops post-harvest to prolong storage[59].

However, the aspect of this that is most interesting is that, as in animals, it is in the mediation of stress responses that H_2S has been most widely studied. Stresses to which plants have been exposed, and to which H_2S has been shown to have an effect are shown in Table 1, but include salt stress[53,60] and heavy metal stress[61-66]. Also of interest here is the involvement in pathogen challenge[67-69]. But of particular relevance is that many of these stress responses, both in animals and plants, involve the generation and response to ROS and RNS. Therefore, it can be argued that H_2S is not working in isolation here, but as part of a team that includes ROS and NO.

The influence of H_2S on ROS and RNS signaling

As discussed elsewhere[70], H_2S has a major influence on the metabolism of both ROS and RNS, especially H_2O_2 and NO. Therefore H_2S

will have a profound effect on the signaling that may ensue from the production and perception of ROS and RNS.

In some organisms such as plants, H_2S is present all the time as it is an integral part of sulfate metabolism[23]. But is can also feed into other sulfur metabolism, for example the presence of H_2S can lead to an increase in glutathione[71]. It is not only the presence of glutathione that is important but the reduction state in which it exists that is critical to an understanding of its sphere of influence in cells. This has been argued very cogently[72]. Cells maintain glutathione mainly in the reduced state (GSH) with a low amount of oxidised glutathione (GSSG). However, because the redox state influenced by the GSH/GSSG couple is a squared derivation of the Nernst equation, the overall concentration of glutathione is important, so if H_2S increases the cellular glutathione the redox state of the cell will be altered[72-73]. This will influence the structure and functioning of many proteins and may in some cases lead to apoptosis or necrosis[72]. And it is important not to forget that GSH is a major antioxidant in cells and has a massive influence on H_2O_2 accumulation, and effects, including signaling.

Furthermore, glutathione can lead to the alteration of proteins, with thiol groups undergoing the process of *S*-glutathionylation[74]. This mechanism can have profound effects on the activity of proteins, as discussed further below, driven by altering levels and the redox state of glutathione, perhaps by increased H_2S generation in cells or from environmental exposure.

It is not only glutathione that has been found to be influenced by the presence of H_2S. Other antioxidants have been altered too. Another important compound used for the control of both ROS and cellular redox is ascorbate,

the level of which can be altered by H_2S[75]. However, it is not a given that H_2S will lead to increased antioxidant levels, as reported using pepper plants[53]. Here, the levels of various antioxidants were quite variable, with the concentrations of some increasing and some decreasing. However, the overall trend appeared to be an elevation of levels and therefore supports the notion that H_2S is a protective chemical. This is exemplified by the use of H_2S as a treatment of crops post-harvest, where the levels of antioxidants appeared to mediate the longevity of fruits in storage[59].

As well as having an influence on the molecules which help to control ROS and RNS accumulation, H_2S may have an influence on the ROS and RNS directly. It is known that ROS and NO can react to generate peroxynitrite ($ONOO^-$)[7] and this can react with H_2S[76] with the potential for the creation of new signals. In a similar way NO and H_2S can react together to form nitrosothiols[77]. The direct reactions of H_2S with ROS has been discussed in depth recently[37]. Reactions with superoxide anions, H_2O_2, hypochlorite and hydroxyl radicals, as well as intermediates formed from them such as lipid peroxyl and protein radicals, would be expected. Therefore H_2S could in theory lower the levels of all such ROS in cells, but it has also been argued that the levels of antioxidants are so high that the influence of a low concentration of H_2S would be negligible in this[37]. The fact that H_2S is a relatively weak reductant, especially when it has to be considered that cells have a relatively high concentration of glutathione, has been discussed previously[78]. It also has to be remembered that high levels of H_2S are toxic and will stop mitochondrial respiration[20].

H_2S can also have an effect on the enzymes that generate ROS and NO. One of the families of enzymes that is most influential in the generation of ROS are the NADPH oxidases, in both animals and plants. It has been shown that H_2S donors can modulate the expression of NADPH oxidases in smooth muscle cells[79] and fibroblasts[80].

An interesting observation in plants was the fact that the activity of glucose-6-phosphate dehydrogenase (G6PDH) was increased following H_2S treatment[81]; this is an enzyme which can produce ROS in roots. These experiments were carried out under salt stress, which is also known to be influenced by H_2S[53].

A similar situation exists when looking at NO metabolism. H_2S can influence the activity of the enzyme nitric oxide synthase (NOS)[82], perhaps in an indirect manner[83] or by an up-regulation of the enzyme[84]. However, such data cannot easily be transferred across to plants, as the existence of NOS in plants has not been well established[85]. Having said this, there are many other enzymes that can generate NO, such as nitrate reductase, xanthine oxidase and other redox based enzymes (see recent review[85]). It will be important to establish if any of these is influenced by H_2S, and whether this affects the overall NO metabolism and signaling of cells.

Is compartmentalisation important in H_2S responses?

One of the issues in cell signaling is the notion of compartmentalisation, i.e. the concentration of signaling molecules or their activities are not uniform in organelles or the cytoplasm of the cell. Reports talk of “hotspots” of signals, an idea which has been talked about for calcium

signaling for many years[86]. Hotspots have been discussed for other signaling molecules, and of particular relevance here is the idea that ROS signaling has hotspots[87]. New probes being developed may help to answer such questions for H_2S[88]. However, it is an important question to answer. If H_2S is to feed into signaling it needs to be produced and perceived in the right place, otherwise signaling will not take place. Of relevance to respiratory science, if H_2S is to be used by the mitochondria then it needs to be at a high enough concentration to be useful, but on the other hand not at such a high concentration that inhibition of the Complex IV takes place[20]. Being a gas and reasonably soluble it would be able to diffuse easily through cells and between cells, so the positioning of both the enzymes which generate H_2S, and may be more importantly remove it, will be crucial to any compartmentalisation which is seen. Furthermore, it has been recently argued that published data often shows that the measured concentration of H_2S is much higher than would be expected, or indeed possible, in a physiological context[89]. Therefore, the local concentrations of H_2S in any hotspots that do exist will be very important to determine, as it is not the overall average concentration of H_2S that any downstream signalling component will be exposed to but rather the immediate local concentration, which may differ considerably from any global measurement.

The influence of H_2S on proteins

If H_2S has effects in cells then it would be expected that many of those effects will be mediated by proteins, and as H_2S is a relatively reactive compound then direct alterations of those proteins may be suggested.

Certainly, direct interaction of proteins with both ROS and NO have been widely reported. The susceptible group on protein is often, but not exclusivity the thiol side chain. As can be seen in Figure 2, the thiol (-SH) group can undergo a variety of modifications[4]. Low levels of H_2O_2 can lead to the formation of disulfide bonds in proteins, stabilizing them and potentially altering their activity. Alternatively, lone thiols may be oxidized to the sulfenic acid form. Higher H_2O_2 can lead to the formation of the sulfinic acid form, while higher H_2O_2 still will lead to the formation of the sulfonic acid group. Lower oxidation states are thought to be reversible while the sulfonic acid formation is an irreversible step. Alternatively, NO will lead to S-nitrosylation and again a change in the activity of the protein may be the result. The formation of such NO modification is also reversible[4]. Also, as mentioned above, thiols may be modified in the presence of glutathione[74], a compounds which itself may be under the influence of the accumulation of H_2S[71].

Recently it has been found that thiol modifications of proteins may also occur in the presence of H_2S. One such protein was found to be a transcription factor, NF-kB[38]. Therefore, in this case the H_2S could have a direct effect on gene expression without the need to modulate the activity of a cell's signaling transduction system. However, the fact that this thiol may be modified opens the door for the potential for a large swage of similar modifications. It has already been reported that H_2S can modify proteins in plants[90].

Proteins such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH) are known, for example, to be modified by both H_2O_2 and NO[91-92], and in that particular case the enzyme is translocated to the nucleus where it

can alter gene expression. It is not unlikely that the thiols on such enzymes are also open to modification by H_2S. Other examples of modification by the presence of H_2O_2 include SAM synthetase (S-adenosylmethionine synthetase), alcohol dehydrogenase and glutamine synthetase[91], while methods such as those of Jaffrey & Snyder[93] have revealed a wide range of proteins which may undergo *S*-nitrosylation. Examples of such studies are those by Grennan[94] and Lindermayr *et al*.[95]. Proteins which are seen to undergo *S*-nitrosylation have a wide range of functions but include those involved in metabolism, signaling responses and following stress. A quick look through the lists of NO and ROS modified thiols shows a large overlap, and therefore if such thiols are exposed and accessible to H_2O_2 and NO then it could be assumed perhaps that they are accessible to H_2S too and therefore sulfhydration of these proteins, with a change in activity perhaps, is likely. Perhaps more importantly, as argued recently[70], if these thiols are being modified by H_2S then they are no longer accessible to be modified by H_2O_2 and NO, so the capacity for such signaling may well be severely altered in the presence of H_2S. Obviously, the relative reactivity of the thiols to NO, ROS and H_2O_2 and the relative concentrations of these compounds needs to be taken into account before a full understanding of this likely competition for thiol modifications is understood.

A look through the literature could identify other proteins as likely targets too. Both NO and H_2O_2 modify the activity of important signaling proteins such as mitogen-activated protein kinases (MAPKs[96-97]). For example in plants both MAPK3 and MAPK6 are activated by reactive signaling components such as ROS, and therefore it is not unlikely that H_2S has an

effect here too, which again could either have a direct effect on signaling, leading to a response, or interfere with the signaling mediated by NO and ROS.

Although not relevant to mammals, signaling proteins involved in the two-component systems are also susceptible to H_2O_2. H_2O_2 sensing in plants has been found to involve ETR1[98], an ethylene receptor, as well as other histidine kinases[99]. Once again, there is little reason not suspect that H_2S may also have an effect here. Of particular relevance to respiratory physiology, such receptors have been found to be able to mediate stomatal aperture movements and therefore the transpiration stream in plants. As outlined above, it is already known that H_2S causes stomatal opening in many cases[51-53], while it has been reported to cause stomatal closure by others[49-50]. Therefore, the proteins affected by H_2S in such systems will be important to identify.

Tools for the study of H_2S signaling

If future work is to study the physiological and molecular effects of H_2S then there is a need for some tools to facilitate this. A critical discussion of this topic has been published by Olson.[78] H_2S is a gas so it is possible to add it directly to an organism or samples but this is not always easy. Compounds such as sodium hydrosulfide (NaSH) or sodium sulfide (Na_2S), which generate H_2S rapidly into solution are available. However, with the use these compounds H_2S may be present transiently and rapidly move to the vapour phase, necessitating their use in high concentrations. To overcome this, a new range of compounds has been developed (and these are now on the market), which enable the release of H_2S into solution with more appropriate

'release' kinetics and even targeted to specific organelles such as mitochondria[100-102], a predominant source of intracellular ROS, and these compounds have been used in a range of organisms including plants[49-53].

As well as a need to supply H_2S, methods to measure H_2S in solution are also required if its actions in cells are to be understood.. Established methods for doing this include those based on methylene blue,[103] although other technologies are available. Sensors can be used, for example, to measure H_2S in the gaseous phase around organisms[104], or in breathe from animals. However, the validity of methods to measure H_2S have recently been very well discussed[89] with the view that some data published indicates that H_2S is at a much higher concentration that would either be expected or would be physiological. It will be important for future work to take such arguments into account.

These global methods also give no information about where in cells the H_2S may be, and besides, if the H_2S is being measured outside of the plant it begs the question as to its physiological relevance. To overcome some of these drawbacks fluorescent probes have been developed[88] and the use of these should be able to reveal which cells produce H_2S, when it is produced and whether its concentration is uniform in cells and tissues. Future work with improving ways to deliver and measure H_2S will no doubt shed light on how it is involved in cellular events and cell signaling.

Conclusion

It is now well known that many reactive compounds including ROS, NO and H_2S are instrumental in the control of cellular functions. H_2S has an impact on

respiratory physiology, from having an effect on the gills of zebrafish[32], to controlling stomatal opening and closing on plants[49-53], to the contribution, or indeed inhibition, of mitochondrial function[20-22]. However, the accumulation and the effects of H_2S should not be considered in isolation. It is clear that H_2S will have a major impact on the metabolism of both ROS and RNS. It may have this effect by partaking in direct reactions, by altering levels of antioxidants or in a competition for thiol groups on proteins. What is becoming clear is that H_2S is an important molecule, the basis of which may form future drug therapies[105-107], or help in crop protection and storage[59].

References

1. Palmer, R.M.J., A.G. Ferrige & S. Moncada. 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **327**: 524 – 526.
2. Neill, S.J., R. Desikan, A. Clarke *et al*. 2002. Hydrogen peroxide and nitric oxide as signalling molecules in plants. *J. Exp. Bot*. **53**: 1237-1247.
3. Hancock, J.T. 2009. The role of redox mechanisms in cell signaling. *Molecular Biotechnology* **43**: 162-166.
4. Wilson, I.D., S.J. Neill & J.T. Hancock. 2008. Nitric oxide synthesis and signalling in plants. *Plant, Cell and Environment* **31**: 622-631.
5. Ku, H.-H., U.T. Brunk & R.S. Sohal. 1993. Relationship between mitochondrial superoxide and hydrogen peroxide production and longevity of mammalian species. *Free Radical Biology and Medicine* **15**: 621-627.

6. Turrens, J.F. 2004. Mitochondrial formation of reactive oxygen species. *The Journal of Physiology* **552**: 335-344.

7. Klotz, L.O., P. Schroeder & H. Sies. 2002. Peroxynitrite signaling: receptor tyrosine kinases and activation of stress-responsive pathways. *Free Radical Biology and Medicine* **33**: 737-743.

8. Bright, J., R. Desikan, J.T. Hancock *et al*. 2006. ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H_2O_2 synthesis. *Plant Journal* **45**: 113-122.

9. McInnis, S.M., R. Desikan, J.T. Hancock *et al*. 2006. Production of reactive oxygen species and reactive nitrogen species by angiosperm stigmas and pollen: potential signalling crosstalk? *New Phytologist* **172**: 221-228.

10. Wang, R. 2002. Two's company, three's a crowd: can H_2S be the third endogenous gaseous transmitter? *The FASEB Journal* **16**: 1792-1798.

11. Wang, R. 2003. The gasotransmitter role of hydrogen sulfide. *Antioxidants & Redox Signaling* **5**: 493-501.

12. Mustafa, A.K., M.M. Gadalla & S.H. Snyder. 2009. Signaling by gasotransmitters *Science Signaling* ***2***: re2.

13. Hancock, J.T., M. Lisjak, T. Teklic *et al*. 2011. Hydrogen sulfide and signaling in plants. *CAB Reviews: Perspectives in Agriculture, Veterinary Science. Nutrition and Natural Resources* **6**: 1-7.

14. Li, L., P. Rose & P.K Moore. 2011. Hydrogen sulfide and cell signaling. *Annu. Rev. Pharmacol. Toxicol.* **51**: 169–187.

15. Wang, R. 2012. Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. *Physiol. Rev*. **92**: 791–896.

16. Kimura, H. 2013. Physiological function of hydrogen sulfide and beyond. *Nitric Oxide* **31**: S11.

17. Kolluru, G.K., X. Shen, S.C. Bir *et al*. 2013. Hydrogen sulfide chemical biology: Pathophysiological roles. *Nitric Oxide* **35**: 5-20.

18. Li, Z.G. 2013. Hydrogen sulfide: a multifunctional gaseous molecule in plants. *Russ. J. Plant Physiol*. **60**: 733-740.

19. Hancock, J.T. 2010. *Cell Signalling*. Oxford University Press, UK, Oxford.

20. Dorman, D.C., F.J. Moulin, B.E. McManus, *et al*. (2002) Cytochrome oxidase inhibition induced by acute hydrogen sulfide inhalation: correlation with tissue sulfide concentrations in the rat brain, liver, lung, and nasal epithelium. *Toxicology Sciences* **65**: 18-25.

21. Bouillaud, F., C. Ransy & M. Andriamihaja. 2013. Sulfide and mitochondrial bioenergetics. *Nitric Oxide* **31**: S15.

22. Helmy, N., C. Prip-Buus & C. Vons *et al*. 2014. Oxidation of hydrogen sulfide by human liver mitochondria. *Nitric Oxide* **41**: 105-112.

23. Calderwood, A. & S. Kopriva. 2014. Hydrogen sulfide in plants: From dissipation of excess sulfur to signaling molecule. *Nitric Oxide* **41**: 72-78.

24. Martin, W., J. Baross, D. Kelley, *et al*. 2008. Hydrothermal vents and the origin of life. *Nature Reviews Microbiology* **6**: 805-814.

25. Clarke, P.H. 1953. Hydrogen sulfide production by bacteria. *Journal of General Microbiology* **8**: 397-407.

26. Mittler, R. S. Vanderauwera, N. Suzuki, *et al*. 2011. ROS signalling: the new wave? *Trends on Plant Science*, **16**: 300-309.

27. Szinicz, L. 2005. History of chemical and biological warfare agents. *Toxicology* **214**: 167-181.

28. CBS News. 2014. Rescuers forced to halt efforts on Japan volcano. 29 September 2014, Accessed 29 October 2014. http://www.cbsnews.com/news/japans-mount-ontake-volcano-rescue-efforts-delayed-hydrogen-sulfide-gas-levels-hazardous/

29. Wientjes, F.B. & A.W. Segal. 1995. NADPH oxidase and the respiratory burst. *Seminars in Cell Biology* **6**: 357-365.

30. Fang, F.C. 2004. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. *Nature Reviews Microbiology* **2**: 820-832.

31. Miller DL, Roth MB (2007) Hydrogen sulfide increases thermotolerance and lifespan in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* **104**: 20618-20622.

32. Porteus, C.S., S.J. Abdallah, J. Pollack *et al*. 2014. The role of hydrogen sulfide in the control of breathing in hypoxic zebrafish (*Danio rerio*). *The Journal of Physiology* **592**: 3075-88.

33. Prabhakar, N.R. 2012. Carbon monoxide (CO) & hydrogen sulfide (H_2S) in hypoxic sensing by the carotid body. *Respiratory Physiology and Neurobiology* **184**:165-169.

34. Alvarez, C., L. Calo, L.C. Romero, *et al*. 2010. An *O*-acetylserine(thiol)lyase homolog with L-cysteine desulfhydrase activity regulates cysteine homeostasis in Arabidopsis. *Plant Physiology* **152**: 656-669.

35. Youssefian, S., M. Nakamur & H. Sano. 1993. Tobacco plants transformed with the O-acetylserine (thiol) lyase gene of wheat are

resistant to toxic levels of hydrogen sulfide gas. *The Plant Journal* **4**: 759-769.

36. Tai, C.H. & P.F. Cook. 2000. O-acetylserine sulfhydrylase. *Advances in Enzymology and Related Areas of Molecular Biology* **74**: 185-234.

37. Li, Q. & J.R. Lancaster Jr. 2013. Chemical foundations of hydrogen sulfide biology. *Nitric Oxide* **35**: 21-34.

38. Sen, N., B.D. Paul, M.M. Gadalla, *et al*. 2012. Hydrogen sulfide-linked sulfhydration of NF-κB mediates its antiapoptotic actions. *Molecular Cell* **45**: 13-24.

39. Polhemus, D.J., D.J. Lefer. 2014. Emergence of hydrogen sulfide as an endogenous gaseous signaling molecule in cardiovascular disease. *Circulation Research* **114**: 730-737.

40. Whiteman, M., K.M. Gooding, J.L. Whatmore, *et al*. 2010. Adiposity is a major determinant of plasma levels of the novel vasodilator hydrogen sulfide. *Diabetologia* **53** :1722-1726.

41. Mani, S., H. Li, A. Untereiner, *et al*. 2013. Decreased endogenous production of hydrogen sulfide accelerates atherosclerosis. *Circulation* **127**: 2523-2534.

42. Liu, Z., Y. Han, L. Li, *et al*. 2013. The hydrogen sulfide donor, GYY4137, exhibits anti-atherosclerotic activity in high fat fed apolipoprotein E(-/-) mice. *Br. J. Pharmacol* **169**: 1795-1809.

43. Ahmed, A. 2013. Can hydrogen sulfide prevent preeclampsia and fetal growth restriction? *Nitric Oxide* **31**: S17.

44. Al-Magableh, M., H. Ng, B. Kemp-Harper, et al. 2013. Hydrogen sulfide protects endothelial function under conditions of oxidative stress. *Nitric Oxide* **31**: S25.

45. Nagpure, B. & J.-S. Bian. 2013. Neuroprotective effect of hydrogen sulfide: Regulation of amyloidosis and inflammation in SH-SY5Y neuroblastoma and BV-2 micro glia cells. *Nitric Oxide* **31**: S22.

46. Wang, R. 2013. A stinky remedy for atherosclerosis. *Nitric Oxide* **31**: S17-18.

47. Dooley, F.D., S.P. Nair & P.D. Ward. 2013. Increased growth and germination success in plants following hydrogen sulfide administration. *PLoS One* **8**: e62048.

48. Li, Z.G., M. Gong & P. Liu. 2012. Hydrogen sulfide is a mediator in H_2O_2-induced seed germination in *Jatropha Curcas*. *Acta Physiol. Plant.* **34**: 2207–2213.

49. García–Mata, C. & L. Lamattina. 2010. Hydrogen sulfide, a novel gasotransmitter involved in guard cell signalling. *New Phytologist* **188**: 977-984.

50. García-Mata, C. & L. Lamattina. 2013. Gasotransmitters are emerging as new guard cell signaling molecules and regulators of leaf gas exchange *Plant Science* **201/202**: 66–73.

51. Lisjak, M., N. Srivastava, T. Teklic, *et al*. 2010. A novel hydrogen sulfide donor causes stomatal opening and reduces nitric oxide accumulation. *Plant Physiology and Biochemistry* **48**: 931-935.

52. Lisjak, M., T. Teklic, I.D. Wilson, *et al*. 2011. Hydrogen sulfide effects on stomatal apertures. *Plant Signaling and Behavior* **6**: 1444-1446.

53. Lisjak, M., T. Teklic, I.D. Wilson, *et al*. (2013) Hydrogen sulfide: Environmental factor or signaling molecule? *Plant, Cell & Environment* **36**: 1607-1616.

54. Liu, J., L.-X. Hou, G.-H. Liu, *et al*. 2011. Hydrogen sulfide induced by nitric oxide mediates ethylene-induced stomatal closure of *Arabidopsis thaliana*. *Chinese Science Bulletin* **56**: 3547-3553.

55. Jin, Z.P., S.W. Xue, Y.N. Luo, et al. 2013. Hydrogen sulfide interacting with abscisic acid in stomatal regulation responses to drought stress in *Arabidopsis*. *Plant Physiol. Biochem*. **62**: 41–46.

56. Zhang, H., J. Tang, X.P. Liu, *et al*. 2009 Hydrogen sulfide promotes root organogenesis in *Ipomoea batatas, Salix matsudana and Glycine max*. *J. Integr. Plant Biol*. **51**: 1084–1092.

57. Lin, Y.-T., M.-Y. Li, W.-T. Cui, *et al*. 2012. Haem oxygenase-1 in involved in hydrogen sulfide induced cucumber adventitious root formation. *Journal of Plant Growth Regulation* **31**: 519-528.

58. Zhang, H., S.L. Hua, Z.J. Zhang *et al*. 2011. Hydrogen sulfide acts as a regulator of flower senescence in plants. *Postharv. Biol. Technol.* **60**: 251–257.

59. Hu, L.-Y., S.-L. Hu, J. Wu *et al*. 2012. Hydrogen sulfide prolongs postharvest shelf life of strawberry and plays an antioxidative role in fruits. *Journal of Agricultural and Food Chemistry* **60**: 8684-8693.

60. Wang, Y., L. Li, W. Ciu *et al*. 2012. Hydrogen sulfide enhances alfalfa (*Medicago sativa*) tolerance against salinity during seed germination by nitric oxide pathway. *Plant and Soil* **351**: 107-119.

61. Zhang, H., L.Y. Hu, K.D. Hu *et al*. 2008. Hydrogen sulfide promotes wheat seed germination and alleviates the oxidative damage against copper stress. *Journal of Integrative Plant Biology* **50**: 1518-1529.

62. Chen, J., W.-H. Wang, F.-H. Wu *et al*. 2012. Hydrogen sulfide alleviates aluminum toxicity in barley seedlings. *Plant and Soil* **362**: 301-318.

63. Zhang, H., Z.Q. Tan, L.Y. Hu, *et al*. 2010. Hydrogen sulfide alleviates aluminum toxicity in germinating wheat seedlings. *Journal of Integrative Plant Biology* **52**: 556-567.

64. Zhang, H., L.Y. Hu, P. Li, 2010 Hydrogen sulfide alleviated chromium toxicity in wheat. *Biol. Plant*. **54**: 743–747.

65. Li, L., Y. Wang & W. Shen. 2012. Roles of hydrogen sulfide and nitric oxide in the alleviation of cadmium-induced oxidative damage in alfalfa seedling roots. *BioMetals* **25**: 617-631.

66. Ali, B., W.J. Song, W.Z. Hu, *et al.* 2014. Hydrogen sulfide alleviates lead-induced photosynthetic and ultrastructural changes in oilseed rape. *Ecotoxicology and Environmental Safety* **102**: 25-33.

67. Bloem, E., A. Riemenschneider, J. Volker, *et al*. 2004. Sulfur supply and infection with *Pyrenopeziza brassica* influence L-cysteine desulfhydrase activity in *Brassica napus* L. *Journal of Experimental Botany* **55**: 2305-2312.

68. Bloem, E., K. Rubekin, S. Haneklaus, et al. 2011. H_2S and COS gas exchange of transgenic potato lines with modified expression levels of enzymes involved in sulfur metabolism. *J. Agron. Crop Sci.* **197**: 311–321.

69. Bloem, E., S. Haneklaus, J. Kesselmeier, *et al*. 2012. Sulfur fertilization and fungal infections affect the exchange of H_2S and COS from agricultural crops. *Journal of Agricultural and Food Chemistry* **60**: 7588-7596.

70. Hancock, J.T. & M. Whiteman. 2014. Hydrogen sulfide and cell signalling: Team player or referee? *Plant Physiology and Biochemistry* **78**: 37-42.

71. De Kok, J.L., W. Bosma, F.M Maas *et al*. 1985. The effect of short-term H_2S fumigation on water-soluble sulfydryl and glutathione levels in spinach. *Plant, Cell and Environment* **8**: 189-194.

72. Schafer, F.Q. & G.R. Buettner. 2001. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radical Biology and Medicine*. **30**: 1191-1212.

73. Hancock, J.T., R. Desikan, S.J. Neill *et al*. 2004. New equations for redox and nano signal transduction. *Journal of Theoretical Biology*, **226**, 65-68.

74. Sun C, Shi Z-Z, Zhou X, Chen L, Zhao X-M (2013) Prediction of *S*-glutathionylation sites based on protein sequences. *PLoS ONE* **8**: e55512.

75. Shan, C.J., S.L. Zhang, D.F Li *et al*. 2011. Effects of exogenous hydrogen sulfide on the ascorbate and glutathione metabolism in wheat seedlings leaves under water stress. *Acta Physiol. Plant*. **33**: 2533–2540.

76. Carballal, S., M. Trujillo, E. Cuevasanta *et al*. 2011. Reactivity of hydrogen sulfide with peroxynitrite and other oxidants of biological interest. *Free Radic. Biol. Med.* **50**: 196-205.

77. Whiteman, M., L. Li, I. Kostetski *et al*. 2006. Evidence for the formation of a novel nitrosothiol from the gaseous mediators nitric oxide and hydrogen sulfide. *Biochemical and Biophysical Research Communications* **343**: 303-310.

78. Olson, K.R. 2012. A practical look at the chemistry and biology of hydrogen sulfide. *Antioxid Redox Signal.* **17**: 32–44.

79. Muzaffar, S., N. Shukla, M. Bond *et al*. 2008. Exogenous hydrogen sulfide inhibits superoxide formation, NOX-1 expression and Rac1 activity in human vascular smooth muscle cells. *Journal of Vascular Research*. **45**: 521-528.

80. Pan, L.L., X.H. Liu, Y.Q Shen *et al*. 2013. Inhibition of NADPH oxidase 4-related signaling by sodium hydrosulfide attenuates myocardial fibrotic response. *International Journal of Cardiology*. **168**: 3770-3778.

81. Li, J., H. Jia, J. Wang *et al*. 2013. Hydrogen sulfide is involved in maintaining ion homeostasis via regulating plasma membrane Na^+/H^+ antiporter system in the hydrogen peroxide-dependent manner in salt-stress *Arabidopsis thaliana* root. *Protoplasma* **251**: 899-912.

82. Kubo, S., Y. Kurokawa, I. Doe *et al*. 2007. Hydrogen sulfide inhibits activity of three isoforms of recombinant nitric oxide synthase. *Toxicology* **241**: 92-97.

83. Kida, M., T. Sugiyama, T. Yoshimoto T *et al*. 2013. Hydrogen sulfide increases nitric oxide production with calcium-dependent activation of endothelial nitric oxide synthase in endothelial cells. *European Journal of Pharmaceutical Sciences* **48**: 211-215.

84. Kondo, K., S. Bhushan, A.L. King *et al*. 2013. H_2S protects against pressure overload-induced heart failure via upregulation of endothelial nitric oxide synthase. *Circulation* **127**: 1116-1127.

85. Hancock, J.T. & S.J. Neill. 2014. NO Synthase? *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*. (*in press*).

86. Bononi, A., S. Missiroli, F. Poletti *et al*. 2012. Mitochondria-associated membranes (MAMs) as hotspot Ca^{2+} signaling units. Calcium Signaling*:* Advances in Experimental Medicine and Biology **740**: 411-437.

87. Figueiredo de Rezende, F., A.M. Lima, S. Niland *et al*. 2012. Integrin α7β1 is a redox-regulated target of hydrogen peroxide in vascular smooth muscle cell adhesion. *Free Radical Biology and Medicine* **53**: 521-531.

88. Lin, V.S., A.R. Lippert & C.J. Chang. 2013. Cell-trappable fluorescent probes for endogenous hydrogen sulfide signalling and imaging H_2O_2-dependent H_2S production. *Proc. Natl. Acad. Sci. U.S.A.* **110**: 7131-7135.

89. Olson, K.R. 2013. Hydrogen sulfide: both feet on the gas and none on the brake? *Frontiers in Physiology* **4**: 2.

90. Romero, L.C., M.A. Aroca, A. Serna *et al*. 2013. Proteomic analysis of endogenous S-sulfhydration in *Arabidopsis thaliana*. *Nitric Oxide* **31**: S23.

91. Hancock, J.T., D. Henson, M. Nyirenda *et al*. 2005. Proteomic identification of glyceraldehyde 3-phosphate dehydrogenase as an

inhibitory target of hydrogen peroxide in *Arabidopsis*. *Plant Physiology and Biochemistry* **43**: 828-835.

92. Holtgrede, S., J. Godlke, J. Starmann *et al*. 2008. Regulation of plant cytosolic glyceraldehyde 3-phosphate dehydrogenase isoforms by thiol modifications. *Physiologia Plantarum* **133**: 211-228.
93. Jaffrey, S.R. & S.H. Snyder. 2001. The biotin switch method for the detection of *S*-nitrosylated proteins. *Sci. STKE* **2001**: pl1.
94. Grennan, A.K. 2007. Protein S-nitrosylation: Potential targets and roles in signal transduction. *Plant Physiology* **144**: 1237-1239.
95. Lindermayr, C., G. Sallbach, J. Durner. 2005. Proteomic identification of S-nitrosylated proteins in Arabidopsis. *Plant Physiology* **137**: 921-930.
96. Kovtun, Y., W.L. Chiu, G. Tena *et al*. 2000. Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proceeding of the National Academy of Sciences USA*. **97**: 2940-2945.
97. Wang, P., Y. Du, Y. Li *et al*. 2010. Hydrogen peroxide-mediated activation of MAP kinase 6 modulates nitric oxide biosynthesis and signal transduction in Arabidopsis. *Plant Cell*. **22**: 2981-2998.
98. Desikan, R., J.T. Hancock, J. Bright *et al*. 2005. A role for ETR1 in hydrogen peroxide signaling in stomatal guard cells. *Plant Physiology* **137**, 831-834.
99. Desikan, R., J. Horák, C. Chaban *et al*. 2008. The histidine kinase AHK5 integrates endogenous and environmental signals in *Arabidopsis* guard cells. *PloSOne*, **3**, e2491 (1-15).

100. Li, L., M. Whiteman, Y.Y Guan *et al*. 2008. Characterization of a novel, water-soluble hydrogen sulfide releasing molecule (GYY4137): New insights into the biology of hydrogen sulfide. *Circulation* **117**: 2351-2360.

101. Szczesny B., K. Módis, K. Yanagi *et al*. 2014. AP39, a novel mitochondria-targeted hydrogen sulfide donor, stimulates cellular bioenergetics, exerts cytoprotective effects and protects against the loss of mitochondrial DNA integrity in oxidatively stressed endothelial cells *in vitro*. *Nitric oxide*, **41**: 120-130.

102. Le Trionnaire, S., Perry, A., Szczesny, B., Szabo, C., Winyard, P. G., Whatmore, J. L., Wood, M. E. & Whiteman, M. . The synthesis and functional evaluation of a mitochondria-targeted hydrogen sulfide donor, (10-oxo-10-(4-(3-thioxo- 3*H*-1,2-dithiol-5-yl)phenoxy)decyl)triphenylphosphonium bromide (AP39). *Med. Chem. Commun.* 2014; 5: 728-736.

103. Moest R.R. 1975. Hydrogen sulfide determination by the methylene blue method *Anal. Chem.*, 1975, **47**: 1204–1205.

104. Costello, B.P., R.J. Ewen & N.M Ratcliffe. 2008. A sensor system for monitoring the simple gases hydrogen, carbon monoxide, hydrogen sulfide, ammonia and ethanol in exhaled breath. *Journal of Breath Research* **2**: 037011.

105. Zhang, Y., Z.-H. Tang, Z. Ren *et al*. 2013. Hydrogen sulfide, the next potent preventive and therapeutic agent in aging and age-related diseases. *Molecular and Cellular Biology* **33**: 1104-1113.

106. Xu, S., Z. Liu & P. Liu. 2014. Targeting hydrogen sulfide as a promising therapeutic strategy for atherosclerosis. *International Journal of Cardiology* **172**: 313-317.

107. Li, L., B. Fox, J. Keeble *et al*.. 2013. 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**: 365-376.

108. Stuiver, C.E.E., L.J. De Kok & P.J.C. Kuiper.1992. Freezing tolerance and biochemical changes in wheat shoots as affected by H_2S fumigation. *Plant Physiology and Biochemistry* **30**: 47–55.

109. Li, Z.G., M. Gong, H. Xie *et al*. 2012. Hydrogen sulfide donor sodium hydrosulfide-induced heat tolerance in tobacco (*Nicotiana tabacum* L.) suspension cultured cells and involvement of Ca^{2+} and calmodulin. *Plant Sci*. **185/186**: 185–189.

110. Li, Z.G., X.J. Ding, & P.F Du. 2013. Hydrogen sulfide donor sodium hydrosulfide-improved heat tolerance in maize and involvement of proline. *J. Plant Physiol*. **170**: 741–747.

111. Li, Z.G., S.Z. Yang, W.B. Long *et al*. 2013. Hydrogen sulfide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (*Zea mays* L.) seedlings. *Plant Cell Environ*. **36**: 1564–1572.

112. Zhang, H., M.F. Wang, L.Y. Hua *et al*. 2010. Hydrogen sulfide promotes wheat seed germination under osmotic stress. *Russ. J. Plant Physiol*. **57**: 532–539.

113. Zhang, H., Y.K. Ye, S.H. Wang *et al*. 2009. Hydrogen sulfide counteracts chlorophyll loss in sweet potato seedling leaves and alleviates oxidative damage against osmotic stress. *Plant Growth Regul*. **58**: 243–250.

Table 1. Some of the stress responses in plants in which hydrogen sulfide is thought to have an influence.

Stress to which plant is exposed.	**References to studies reporting H_2S involvement.**
Aluminium	62-63
Cadmium	65
Chromium	64
Copper	61
Freezing	108
Heat	109-111
Lead	66
Osmotic	112
Oxidative	75, 113
Pathogen challenge	67-69
Salt	53, 60
Water	75

Figure 1. Hydrogen sulfide can have two effects on mitochondrial electron transport chain activity. Firstly it can feed electron in the pathway with a concomitant increase in ATP production, or alternately it can inhibit Complex IV so inhibiting ATP production.

Figure 2. The thiol group of proteins may be covalently modified in a variety of ways, including by NO, H_2O_2 and H_2S. It is likely that this is a completion for the exact end resultant modification, each possibly having a different influence on the protein.