ANTIOXIDANTS & REDOX SIGNALING
Volume 15, Number 2, 2011

DOI: 10.1089/ars.2010.3678

FORUM REVIEW ARTICLE

Mitochondria and Sulfide: A Very Old Story of Poisoning, Feeding, and Signaling?

Frédéric Bouillaud[1] and François Blachier[2]

Abstract

Sulfide is a molecule with toxicity comparable to that of cyanide. It inhibits mitochondrial cytochrome oxidase at submicromolar concentrations. However, at even lower concentrations, sulfide is a substrate for the mitochondrial electron transport chain in mammals, and is comparable to succinate. This oxidation involves a sulfide quinone reductase. Sulfide is thus oxidized before reaching a toxic concentration, which explains why free sulfide concentrations are very low in mammals, even though sulfide is constantly released as a result of cellular metabolism. It has been suggested that sulfide has signaling properties in mammals like two other gases, NO and CO, which are also cytochrome oxidase inhibitors. The oxidation of sulfide by mitochondria creates further complexity in the description/use of sulfide signaling in mammals. In fact, in the many studies reported in the literature, the sulfide concentrations that have been used were well within the range that affects mitochondrial activity. This review focuses on the relevance of sulfide bioenergetics to sulfide biology and discusses the case of colonocytes, which are routinely exposed to higher sulfide concentrations. Finally, we offer perspectives for future studies on the relationship between the two opposing aspects of this Janus-type molecule, sulfide. *Antioxid. Redox Signal.* 15, 379–391.

Introduction

The ability of mammals to metabolize the toxic molecule, sulfide, has long been acknowledged. However, only recently has evidence been available that the sulfide disposal pathway is based on a mechanism that is tightly linked to mitochondrial bioenergetics and shared between many organisms. Important pioneering studies in this area were conducted on animal models adapted to sulfide-rich environments (26). It has been proposed that the evolution of eukaryotes involved a symbiotic relationship based on the reduction of sulfur into H_2S by archaea and the oxidation of H_2S by bacteria (58). Based on our current knowledge of sulfide bioenergetics in mammalian cells, experiments to further explore traces of this ancient metabolic cooperation could be performed, as described below.

Sulfide exists as three different forms: the hydrogenated form H_2S, which is a gas that is soluble in water and lipids, and two anionic forms: hemisulfide HS^- and sulfide S^{2-}, with pKa values equal to 7.04 and 11.96, respectively (71). Salts derived from these ions (NaHS, Na_2S) are currently used as "sulfide donors." The dissolution of a sulfide salt in an aqueous phase leads to equilibration of these different forms (H_2S, HS^-, and S^{2-}), and at a physiological pH, HS^- is the predominant form (15, 34).

The increasing interest in the role of H_2S in mammalian cell biology means it is timely to review the interconnection between the sulfide oxidation pathway and mitochondrial energy metabolism. First, this constitutes a link that could help to unify different aspects of the relevance of sulfide to biology. Second, it imposes constraints on the physiological role of sulfide. And third, it suggests new possibilities concerning the intracellular role/traffic of this small sulfide molecule in its relationships with mitochondria.

The Origin of Sulfide

Volcanism

Volcanism is a natural source of sulfide which sometimes gives rise to environments that are biologically relevant at the earth's surface, such as sulfide-containing water sources. Volcanism is an important source of sulfide in deep-sea oceanic ridges where it is the sole or main source of energy for bacteria in the absence of sunlight (20). The sulfide concentration in the region of volcanic outlets is in the hundreds of micromolar range, decreasing rapidly as the distance from the outlet increases (31).

Anaerobic sulfur metabolism of microorganisms

Marshes represent another environment in which sulfide finds a biological relevance. While the oxygenated surface of the mud is lightly colored, just a few millimeters deeper, the

[1]Institut Cochin, Université Paris Descartes, INSERM-U1016, CNRS-UMR8104, Paris, France.
[2]INRA-CRNH-IdF-AgroParisTech, UMR914, Paris Cedex, France.

mud becomes anoxic, darker, and, when stirred, releases H_2S that is easily recognized by its odor. This sulfide derives from the metabolism of anaerobic bacteria that use oxygenated sulfur-containing molecules as electron acceptors. The sulfide concentration in this case is estimated to be up to hundreds of micromolar (26). While bacteria partition according to their respective metabolisms, motile aerobic organisms or those residing during low tide in this sulfide-rich environment have to face sulfide concentrations that can threaten their aerobic nature. Living on the seashore, the worm *Arenicola marina* has been used as a model organism for animals in this environment, and its adaptation to these conditions has been the subject of studies that led to the definition of the "sulfide oxidation unit", as explained below (27).

Gut

The gut is another environment in which relatively high sulfide exposure is an issue. In the digestive tract, anaerobic bacterial flora produce sulfide. It is important to note that, to the best of our knowledge, the presence of lithotrophic bacteria utilizing H_2S as an electron donor has never been reported in the microbiota of the large intestine (7). The sources of sulfur include sulfur-containing amino acids (methionine and cyst(e)ine), inorganic sulfate, and additives (16, 23). Sulfur-containing amino acids that are fermented by the microbiota originate from proteins that have escaped digestion in the small intestine (14, 21). Although the digestion of most proteins is equally efficient (at $\geq$90%) (2, 9, 22), between 6 and 18 g per day of nitrogenous materials (mainly in the form of peptides) are transferred from the small intestine to the cecum in humans (11, 60). This nitrogenous material is degraded by microbiota in the large intestine and by residual pancreatic proteases; leading to the production of numerous peptides and bacterial metabolites that include H_2S (7, 8). In addition, sulfated polysaccharides such as sulfomucins (a component of the mucin layer with protective effects on the colonic epithelium) are endogenous substrates for H_2S production (24). H_2S levels in the luminal content of the human large intestine have been reported to be between 1.0 and 2.4 m*M* (43). Interestingly, there is a correlation between the level of meat (and thus protein) intake and the level of fecal sulfide excretion (44). However, it is worth emphasizing that a large fraction of sulfide is probably in the bound form in the colonic luminal content. This can be deduced from experiments on feces recovered from human volunteers, which indicated that 8% of total sulfide was found in a free (unbound) form (32), representing a free concentration of $\sim 60\ \mu M$. Fecal components with a largely unknown identity have a high capacity for H_2S binding. In rats, it has been shown that zinc (in the form of zinc acetate) decreased total sulfide levels 5-fold, indicating the potential capacity of this divalent cation for sulfide binding and the formation of insoluble salts (63). The addition of 1.0 m*M* bismuth, iron, and zinc (but not magnesium) to batch cultures seeded with fecal flora reduced H_2S concentration by 90%, 58%, and 57% respectively (46). Because of its lipid solubility (45), H_2S penetrates the biological membranes of colonic epithelial cells (56). In the aqueous phase, and at the pH (typically between 6.3 to 6.6) of the colonic luminal content, a fraction of H_2S remains undissociated and at equilibrium with the hemisulfide anion (HS^-) (42, 71). The luminal brush-border membranes of absorbing colonocytes are covered with a layer of mucus and are in contact with the luminal content that contains sulfide in its free and bound forms. It is not clear whether these colonocyte brush-border membranes totally or partly exclude entry of the hemisulfide anion. Depending on its concentration in a free (unbound) form, sulfide can either be used as an inorganic substrate for energy production by colonic epithelial cells (25) or will act at higher concentrations as a "metabolic troublemaker" by inhibiting mitochondrial cytochrome oxidase (39). It is thus tempting to suggest that the oxidation of sulfide by colonic epithelial cells represents a means of controlling intracellular free sulfide concentrations in order to prevent energy depletion in these cells. In turn, the sulfide oxidation pathway enables the recovery of energy from a compound produced by the microbiota.

Cellular metabolism

Several recent reviews have covered this aspect of H_2S metabolism (34, 35). Briefly, H_2S is derived from sulfur-containing amino acids (62) via the trans-sulfuration pathway. Two enzymes are known to release sulfide: cystathionine ß-synthase (CBS) (61), cystathionine γ-lyase (CSE) (12); a third enzyme, 3-mercaptopyruvate sulfur transferase (3MST) also produces sulfide in the presence of a reductant. This 3MST is thought to be mitochondrial and requires the presence of alpha-ketoglutarate and mercaptopyruvate, which are derived in turn from cysteine via the action of cysteine aminotransferase (35, 59). The rate of sulfide production has been estimated in arterial tissue as ranging from 2 (78) to 8 (80) nmol/min/g fresh weight. During the same studies, values for the heart (78) and ileum (80) were also within these limits (5 and 6.5, respectively). Using a different measurement method (41), values of 0.3 in brain slices or 0.45 in colonic muscle was found; these values were expressed as pmol/min/mg fresh weight and were thus immediately comparable and significantly lower, suggesting either a wide variability in the sulfide production rate as a function of the tissue concerned or a strong influence of the experimental method used to evaluate the sulfide production rate. It should be mentioned that in this latter study (41) the estimation of the maximum sulfide production observed with homogenates in the presence of 10 m*M* cysteine and cofactors (and thus the measurement of enzymatic activities) led to values that were at least ten times higher than those observed in slices of the same tissues. The involvement of some of the enzymes mentioned above was verified, either by the use of inhibitors (41, 80) or, for CSE, by genetic manipulation of the mice (78). For example, the use of inhibitors revealed that the contribution of enzymes to the low level of sulfide release by a liver slice could not be detected (41). If it is approximated that 1 mg is equivalent to 1 μl, then sulfide production would lead to a rise in concentrations expressed in μmol/minute. However, at the level of the whole organism, sulfide release is quite low and the excretion of sulfur occurs predominantly in the oxidized form of sulfate (4).

Toxicity of Sulfide

Inhibition of mitochondrial cytochrome oxidase

Sulfide inhibits cytochrome oxidase (mitochondrial complex IV), which transfers electrons from cytochrome c to oxygen to form water. This is the final and irreversible step in the

mitochondrial respiratory chain. The transfer of electrons in this chain is coupled to proton pumping, creating the mitochondrial membrane potential that drives the phosphorylation of ADP into ATP and thus provides the energy required for cellular activity. Therefore, high concentrations of sulfide severely impair cellular bioenergetics. The effect of sulfide on respiration is comparable to that of cyanide (13). In cell homogenates, $\sim 0.3\,\mu M$ sulfide is sufficient to produce half maximal inhibition of the mitochondrial cytochrome oxidase in human and rat colonocytes (39). However, if living cells are exposed to sulfide, sixty to hundred times higher concentrations ($\sim 20\,\mu M$) are needed in the external medium to achieve half maximal inhibition of cellular respiration. This suggests that the intact plasma membrane and cell integrity are efficient barriers against extracellular sulfide. When these values are compared with the sulfide production rate (see above), it can be concluded that, unless it is removed efficiently, cells would be in jeopardy of sulfide poisoning within minutes.

The sulfide inhibition of cellular respiration is reversible (37, 39). While some cells could survive almost indefinitely using the anaerobic glycolysis pathway and possibly withstand a toxic sulfide concentration for an indefinite period of time, this is not the case for the whole organism in which sulfide exposure can be fatal. Reactive oxygen species (ROS) are produced in the mitochondrial respiratory chain (67). This production is estimated to represent only a few percent of the oxygen consumed. Moreover, mitochondria are equipped with antioxidant enzymes to remove ROS. The production of ROS is enhanced when intermediates in complex I or III of the mitochondrial respiratory chain accumulate in a reduced form. Inhibition of mitochondrial cytochrome oxidase results in the accumulation of upstream complexes in a reduced form and may thus enhance superoxide formation, causing oxidative stress. This process could explain why mitochondrial ROS production is associated with hypoxia: the affinity for oxygen of cytochrome c oxidase is high but is decreased by inhibitors (NO, CO, sulfide) (13). Therefore, even if cytochrome oxidase activity is in excess at a low oxygen concentration (hypoxia), the decrease in cytochrome oxidase activity causes an increase in the reduction status of complexes I and III and promotes the conversion of oxygen into superoxide. However, at the same time, sulfide is a reducing agent that can scavenge ROS (74, 75) and provide protection from oxidative stress, which is sometimes considered to be more deleterious than diminished ATP production during hypoxia. Finally, it has also been proposed that sulfide exposure induces mechanisms that ultimately result in an increase in the availability of the antioxidant molecule, GSH (36).

Sulfide exposure of cells

Studies on the adaptation of *Arenicola marina* to a sulfide-rich environment have concluded that mitochondrial ATP production declines at 15–20 μM sulfide concentrations and is abolished at 50 μM sulfide (70). Essentially the same conclusions have been reached with respect to mammalian cells (37). These values for cytochrome oxidase inhibition were obtained using air-saturated aqueous media (*i.e.*, containing 200 μM oxygen), while the oxygen concentration in tissues is considerably lower ($<20\,\mu M$). While a few studies have claimed that the physiological sulfide concentration is extremely low (18, 29, 51, 69, 76), influential reports have also suggested that sulfide concentrations may reach 40 μM (78) or higher (1), as reviewed in (15). The pharmacological use of sulfide in experimental models is usually within the micromolar to millimolar concentration range (64). On the grounds of sulfide sensitivity of cytochrome oxidase, it appears that extracellular free sulfide concentrations of 40 μm or higher are not compatible with mitochondrial function. The discrepancy between estimates of tissue sulfide contents is thought to result from the fact that some of the experimental procedures used to assay sulfide caused a release of sulfide derived from sulfur atoms linked to proteins: iron sulfur centers, sulfane sulfur, etc. (29, 69), thus increasing the risk that these values have no physiological relevance (34). Nonetheless, the sensitivity of cytochrome oxidase to sulfide questions some of the interpretations made when concentrations of 40 μM or higher are used as pharmacological agents (see below).

Neutralizing Sulfide

One strategy to neutralize sulfide may be to bind it to other sulfur atoms (sulfane sulfur, persulfides) or to proteins. This strategy is used by animals living in sulfide-rich environments and involves respiratory pigments (3). The release of acid-labile sulfur from mammalian cell tissues (69) may result from the attack on functional sulfur-containing groups in proteins, such as iron sulfur centers (34), but it may also reflect a pool of bound sulfur that is generated to protect cells from their endogenous sulfide production rate (29, 68,

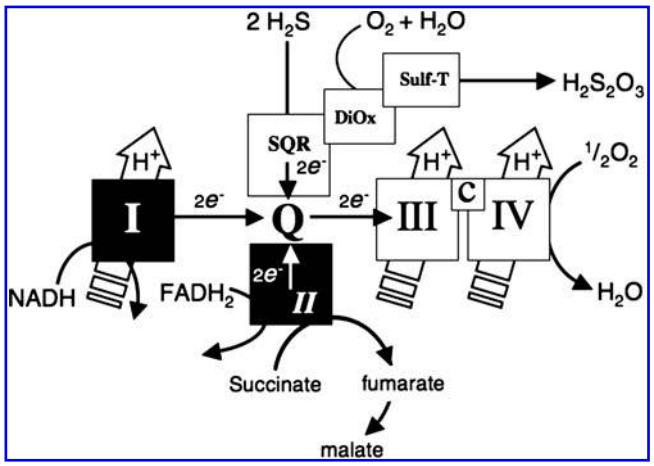


FIG. 1. The mitochondrial respiratory chain and the sulfide oxidation unit. The mitochondrial respiratory complexes (I–IV) are shown. Proton pumping by complexes I, III, and IV is schematized. SQR is shown to be associated with the dioxygenase (DiOx) and sulfur transferase (Sulf-T) that are necessary for the conversion of sulfide into thiosulfate ($H_2S_2O_3$). This figure depicts how the different electron donors (complex I, $FADH_2$ enzymes, complex II, and SQR) converge on coenzyme Q. No topological information is associated with this scheme. The oxidation of two H_2S molecules results in the consumption of one atom of oxygen at the level of complex IV and one molecule of dioxygen (O_2) at the level of the dioxygenase. Hence, two electrons coming from two molecules of sulfide implicate the consumption of three atoms of oxygen, while two electrons from other sources (NADH, $FADH_2$, Complex II) require only one. This results in a 0.75 stoichiometry of O_2/H_2S ($1.5(O_2)/2H_2S$).

69). If sulfide is bound reversibly, its release from intracellular stores remains a possibility. On the other hand, the destruction of sulfide guarantees its irreversible disposal. Sulfide reacts spontaneously with oxygen and forms precipitates with metallic ions (see examples above). These nonenzymatic processes are unlikely to be of importance to the regulation of intracellular sulfide concentrations. Rather, mammalian cells use an enzymatic pathway to destroy sulfide (37), which is especially important in environments such as colonic epithelial cells where sulfide exposure is high (19, 25, 37).

Sulfide as a Mitochondrial Substrate

The mitochondrial catabolism of sulfide has been shown to occur in different organisms. In *A. marina*, the sulfide bioenergetic pathway has been relatively well characterized (27), unlike the case in vertebrates. Avian mitochondria have been reported to produce ATP from sulfide, but this analysis was not quantitative (79). More recent reports have presented a quantitative analysis of sulfide oxidation (25, 37). First, the mechanism involved appeared to be identical to that evidenced in *A. marina* (Fig. 1). Second, a large majority of the cell lines studied revealed significant sulfide oxidation capacity. Sulfide oxidation was also observed with mitochondria isolated from the heart, liver, or kidney of mice, a noticeable exception being the neural tissue because neither of the two neuroblastoma cell lines studied, nor mitochondria from mouse brain, displayed any detectable sulfide oxidation activity (37). Third, the sulfide oxidation pathway has a high affinity for sulfide. In fact, experimental limitations only enabled us to determine the lowest concentration of sulfide with which we could demonstrate sulfide oxidation, which overestimated the threshold (if it exists) above which sulfide oxidation by sulfide quinone reductase (SQR) could take place. When studying permeabilized cells, we deduced that oxidation (or in fact mitochondrial energization) took place at a sulfide concentration of 100 nM or lower (25). In intact cells, it seems that a minimal concentration close to 1 μM in the external medium was necessary to reproducibly detect the oxygen consumption associated with sulfide oxidation (37). Taking into account the ratio of concentrations (60–100 times) observed previously as being reflective of the intactness/permeabilization of the plasma membrane (see above), it suggests that sulfide oxidation can be detected when the intracellular concentration of sulfide, near mitochondria, rises above 10–20 nM, thus further lowering this "threshold value" by comparison with the previous estimate ($<$100 nM) obtained using permeabilized cells. Thus, sulfide oxidation could occur at concentrations where cytochrome oxidase is not inhibited.

In summary, in air-saturated media ($\sim$200 μM oxygen), when the extracellular sulfide concentrations are lower than 1 μM, the fate of sulfide remains open to conjecture. If the concentration ranges between 1 and 10 μM, sulfide can be considered as a mitochondrial substrate for cells equipped with SQR, which is found widely in mammalian tissues (37). Above 10 μM, sulfide becomes toxic and impairs mitochondrial

FIG. 2. Evidence for sulfide oxidation in living cells. *Top:* Tracings that show the oxygen consumption rate (first derivative of the oxygen concentration measured with an Oxygraph) in the absence or presence of sulfide in CHO cells in suspension. The *black line* corresponds to cells poisoned with rotenone, so that only the oxidation of sulfide was recorded. The *gray line* corresponds to cells without any addition, so that sulfide oxidation occurred at the same time as that of other "normal" mitochondrial substrates. This figure is adapted from (37).

Bottom: Diagrams illustrating different states of the mitochondrial sulfide oxidation pathway according to the different additions. The sulfide oxidation unit has been reduced to the SQR while the complete reaction equation is shown. However, for clarity, full reaction schemes have been omitted in **(D)**.

Four sequences of sulfide additions are shown and result in periods during which sulfide oxidation occurs **(A–D)**. While **(D)** follows the single direct addition of a sulfide bolus that immediately increases the concentration in the medium, the others **(A–C)** correspond to periods of sulfide infusion at different rates. **(A)** and **(B)** Sulfide infusion rates match sulfide oxidation rates so that a steady state is obtained with the following characteristics: i) following cessation of the infusion, cells return immediately to their original oxygen consumption rate (without sulfide); ii) virtually no sulfide is present in the medium as it is avidly consumed by SQR within cells, and iii) there is a linear relationship between the sulfide infusion rate and the increase in oxygen consumption. **(C)** The infusion rate is further increased and is now higher than the maximal rate for cellular oxidation. Linearity is lost, and sulfide accumulation in the medium means that sulfide oxidation continues after cessation of the infusion; no steady state is observed and the oxygen consumption rate fluctuates continuously because the rise in sulfide concentration has led to inhibition of the mitochondrial respiratory chain, which is maximal at the time the infusion is halted and gradually disappears thereafter. **(D)** The single addition of sulfide results immediately in a drop in the oxygen consumption of cells (in the absence of rotenone). Over time, the cells recover a subnormal respiratory rate. In the presence of rotenone, although an inhibition of oxygen consumption is clearly visible (compare **B** and **D**), sulfide triggers a rise in oxygen consumption. The difference in the time required for sulfide disposal (as judged from the return to the previous respiratory rate) is affected by the presence/absence of rotenone, thus illustrating the competition between mitochondrial complex I and SQR. In situations **A–C,** the oxidation of sulfide is as fast in the absence of rotenone (*gray trace*). This indicates that "normal" oxidation of carbon-containing substrates is not an obstacle to sulfide oxidation. In this case, the increase in oxygen consumption associated with sulfide infusion is lower because this increase reflects only the activity of the dioxygenase since the total electron transfer in the mitochondrial respiratory chain is unchanged (see Fig. 1).

It should be noted that although the quantity of sulfide added was the same in **B**, **C**, and **D**, the results differ in terms of cellular oxygen consumption rates and the duration of the sulfide oxidation period.

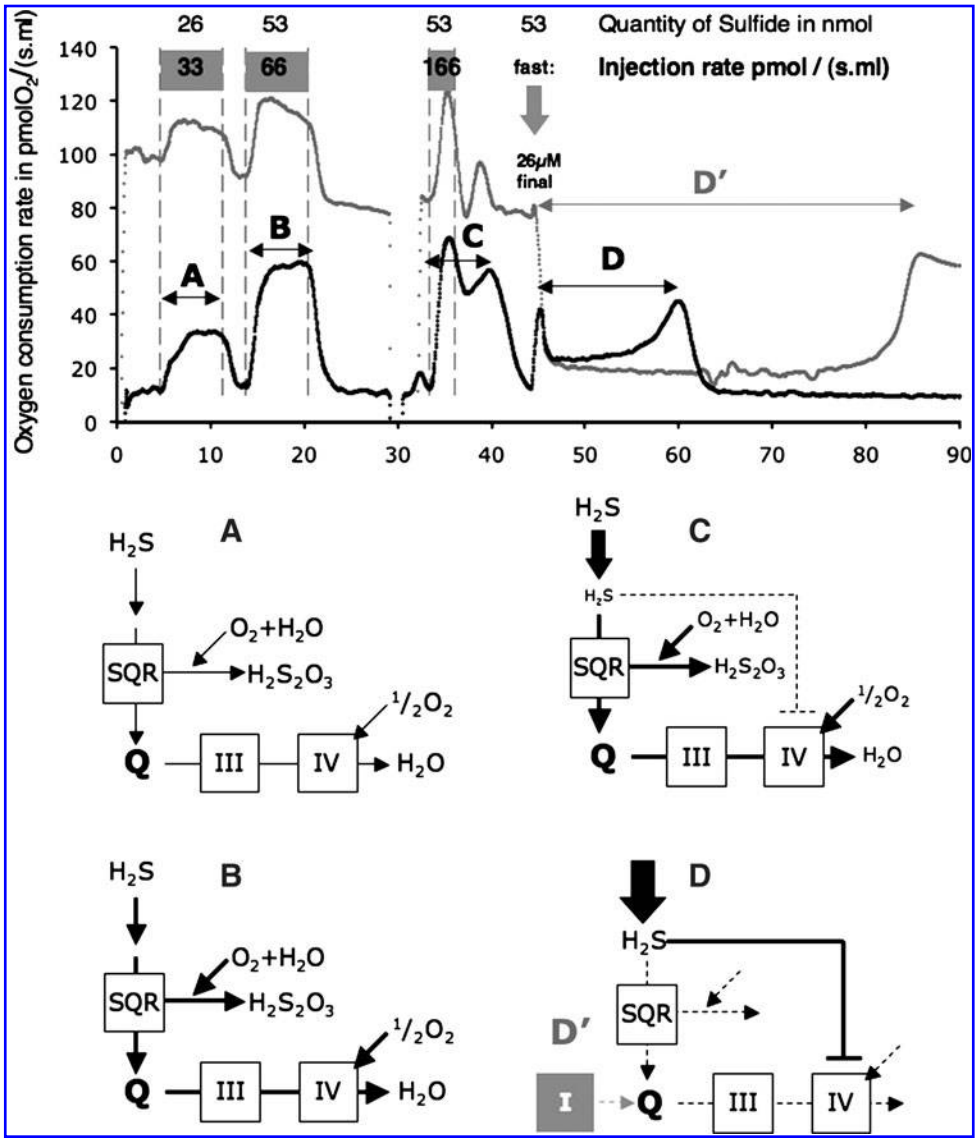
bioenergetics because of the inhibition of cytochrome oxidase, even though the oxidation of sulfide continues.

Methodological issues

One important issue when studying sulfide catabolism in cells or mitochondria is its mode of delivery (Fig. 2). To observe a steady state of sulfide oxidation, sulfide must be infused continuously at a rate that matches its oxidation by cells or isolated mitochondria (25, 37). This has two consequences: first, all sulfide supplied to the cell preparation is immediately oxidized, a conclusion derived from the observation that when sulfide infusion ceases, the oxygen consumption of the cells decreases immediately (Fig. 2, steps A and B). Second, the concentration of sulfide in the medium remains low (but is not precisely known). As a consequence, the flux of sulfide through the oxidation pathway is derived directly from the infusion rate. Under these conditions, the oxidation product, thiosulfate, accumulates in the medium, suggesting that this product is less toxic than sulfide. The administration of a toxic concentration of sulfide (26 μM) immediately impairs cellular respiration, although over time, the sulfide is destroyed and respiration can resume (37, 39) (Fig. 2, step D). Thus, the concentrations of sulfide used during pharmacological experiments (such as those reviewed in (64)) can be expected to be transient.

SQR injects electrons from sulfide into the mitochondrial respiratory chain

Experiments performed with permeabilized colonic epithelial cells demonstrated that sulfide infusion was accompanied by the oxygen consumption and energization of mitochondria (25). It was thus concluded that electrons from sulfide enter into the mitochondrial respiratory chain that couples redox reactions to proton pumping (Fig. 1). Through the use of specific inhibitors, it was demonstrated that electrons from sulfide enter downstream of complex I (which oxidizes NADH and reduces quinone) and upstream of complex III (which oxidizes quinone and reduces cytochrome c). Thus, two electrons from sulfide reduce quinone and are transferred from quinone to complex III cytochrome c and finally reduce



oxygen to water at the level of complex IV (cytochrome oxidase), which is also the target of sulfide toxicity. The entry of electrons from sulfide into the mitochondrial respiratory chain is therefore similar to that of succinate, a substrate commonly used with mitochondrial preparations.

A sulfide oxidizing unit

By comparing the rate of sulfide oxidation with the rate of oxygen consumption, it is possible to calculate a stoichiometry for oxygen/sulfide. The stoichiometry for the re-oxidation of NADH or $FADH_2$ is 0.5 because each molecule of reduced cofactor donates two electrons to the respiratory chain, which in turn reduces a single atom of oxygen (half a molecule of O_2). In contrast, when sulfide is oxidized, the stoichiometry is $\sim$0.75. This value indeed agrees with the reaction scheme proposed for sulfide oxidation releasing thiosulfate (Fig. 1) (27). Thus, the immediate oxidation of sulfide does not generate the final excreted oxidation product, sulfate (4) and further oxidation steps are required. It is still not known if this reflects true compartmentalization or whether the mandatory design of oxygen consumption experiments with a large external volume allowed thiosulfate to leak out sufficiently quickly to escape this further oxidation step within the same cells/mitochondria. In this scheme (27), the membrane-bound mitochondrial enzyme SQR catalyzes the first step in sulfide detoxification, resulting in the synthesis of persulfide (S-SH groups on SQR itself). Then, a sulfur dioxygenase oxidizes the persulfide to sulfite while consuming molecular oxygen and water. Disruption of the mitochondrial sulfur dioxygenase, ETHE1, in mice leads to lethality at between 5–6 weeks after birth because of sulfide toxicity (66), thus indicating the importance of ETHE1 to sulfide disposal. Finally, rhodanese, which is a sulfur transferase, is a good candidate for the conversion of sulfite into the product, thiosulfate. Comparison of the two oxygen/reductant stoichiometries (0.5 and 0.75) is somewhat misleading with regard to the oxygen consumption linked to sulfide oxidation because two sulfide molecules are involved (Fig. 1). A careful examination of the reaction scheme indicates that for the same flux of electrons in the mitochondrial respiratory chain, sulfide oxidation requires three times more oxygen than the oxidation of carbon-derived substrates.

Energy coupling

While mitochondrial energization during sulfide oxidation has been demonstrated, it is more difficult to obtain evidence for ATP synthesis with permeabilized cells (25). However, when experiments were performed with intact cells, the addition of oligomycin, an inhibitor of mitochondrial FoF1 ATP synthase, resulted in a significant decrease in the cellular respiratory rate in the presence of sulfide (37). This establishes that sulfide oxidation is coupled to ATP synthesis. Studies in permeabilized cells have demonstrated that sulfide *per se* does not lead to increased permeability of the mitochondrial inner membrane for protons, and therefore does not cause "uncoupling" (25).

Competition between sulfide and organic mitochondrial substrates

Experiments with cells have enabled an evaluation of the efficiency of sulfide oxidation in the presence of organic substrates (carbohydrates, amino acids, fatty acids). These studies demonstrated that the oxidation of sulfide took priority over the oxidation of carbon-based substrates (37) because SQR injects electrons from sulfide irrespective of the oxidation of other substrates, and the total rate of electron transfer in the respiratory chain remains unchanged. This suggests that sulfide competes efficiently with other electron donors: NADH or $FADH_2$ derived from the oxidation of carbon-based substrates. The efficacy of sulfide as a substrate for oxidative phosphorylation guarantees its efficient removal, thus preventing the poisoning of cytochrome oxidase. Cells therefore appear to be capable of dealing with a continuous influx of sulfide, as long as the maximum rate of sulfide oxidation is not reached (37) and (Fig. 2, situations A and B).

Maximum sulfide oxidation rates and tolerance to sulfide

The maximum sulfide oxidation rate is determined primarily by two factors: 1) the activity of the sulfide oxidation unit with a prominent role of SQR (37), and 2) the activity of the mitochondrial respiratory chain. In experiments where sulfide oxidation is being studied, it is therefore relevant to compare the sulfide delivery rate to the mitochondrial respiratory activity of the cellular/mitochondrial preparation before the initiation of sulfide infusion. The (sulfide infusion rate)/(oxygen consumption rate in the absence of sulfide) ratio is a dimensionless number that yields an estimate of "relative sulfide exposure". Based on our studies (37), most of the cell lines studied tolerated a sulfide exposure of 0.5 (for example in Fig. 2, the CHO cells before sulfide infusion showed a respiratory rate of $\sim$100 then in situation A, the relative sulfide exposure was $\sim$0.33, $\sim$0.66 in B, and $\sim$1.66 in C). Few cell types accepted values higher than 2 (colonocytes, monocytes, myoblasts). To comply with the reaction scheme (Fig. 1), a value of 4 appears to be the maximum theoretical value for this relative sulfide exposure (37), resulting in a three-fold increase in the oxygen consumption rate. These values were approached using a cell line derived from human monocytes (37).

It is also possible to estimate the relative sulfide exposure of tissues by comparing sulfide production rates (see above) with the respiratory rate of the same tissue. The oxygen consumption of the mouse beating heart was within the range 1–10 μmol/min/g fresh weight (28); with a sulfide production rate of 5 nmol/min/g (78), this produces a relative sulfide exposure lower than 0.005. Experiments with mitochondria isolated from mouse liver, heart, and kidney demonstrated that they oxidized sulfide, and from the data shown in (37) it could be estimated that they withstood a relative sulfide exposure of 0.2. In conclusion, the sulfide oxidation capacity of the heart mitochondria appears to be much higher than the estimated sulfide production rate. It is likely that this is also the case with liver and kidney, as it is highly improbable that the sulfide production rates of these organs would reach a value close to 20% of the respiratory rate. The consumption of oxygen dictated by the sulfide production rate can also be calculated by the use of the stoichiometric factor: (oxygen consumption) = 0.75 $\times$ (sulfide production), and led to the same conclusion (37).

Importantly, sulfide oxidation could not be detected in mouse brain mitochondria, or in the two neuroblastoma cell

lines examined (37). Thus, sulfide oxidation appears to be present in many tissues but not in neural tissue, which could therefore be expected to display a lower tolerance of sulfide exposure.

SQR renders sulfide highly unstable in vivo

Thus, with the noticeable exception of neural tissue (brain), the sulfide oxidation unit is found in all the major oxidative organs of the body, and in many cell types (37). Moreover, its sulfide oxidation activity largely exceeds all the estimates of sulfide production rates. Consequently, the expected concentration of free sulfide within tissues would tend towards zero or to the hypothetical threshold necessary to initiate SQR activity. In fact, the evidence for such a threshold remains scarce, although an estimate of its maximum value was determined by our studies (10–20 n*M* within cells and 0.5–1 μM in the external medium). Therefore, unless mitochondrial respiration is poisoned by sulfide, its administration will result in a very short time in concentrations that are lower than these values. Our results are therefore fully consistent with reports suggesting very low sulfide concentrations in plasma and tissues, and provide a mechanistic explanation for these low values.

In articles providing estimates of sulfide production rates (see above), no information was given regarding specific measures designed to protect sulfide from mitochondrial oxidation within the organ/cells studied. One study (41) compared intestinal muscle, brain, and liver. It is interesting to note that sulfide release was detected with brain but not liver slices, even though the intensity of the enzymatic activities thought to generate sulfide had been reported to be higher in the liver. The presence of the sulfide oxidation unit in liver mitochondria but not in brain mitochondria (37) may explain this observation. Consequently, because sulfide is thought to play a physiological role (see below), it could be proposed that the conversion of sulfide into a bound form may also be designed to protect the sulfide from oxidation by SQR, and not just to protect cytochrome oxidase from sulfide toxicity.

Bioenergetics and the Signaling Role of Sulfide

In the cardiovascular system, sulfide apparently exerts vasorelaxant effects that are comparable to those of NO (34, 72). Thus, sulfide (or NO or CO) improves tissue oxygenation and lowers blood pressure. Given the prevalence of high blood pressure and its associated cardiovascular risk, there is considerable interest at present in these gaseous messengers. The disruption of mouse CSE, a sulfide-producing enzyme, results in an elevation of blood pressure (78). While NO is subject to reaction with superoxide forming peroxynitrite (65), a strong oxidant, it is thought that sulfide moderates oxidative stress (see above). Interestingly, sulfide donors have been characterized in some foods. For instance, the cardioprotective effects of garlic appear to be associated with the release of sulfide from diallyl sulfide (6). Sulfide is reported to have anti-inflammatory effects, while excessive sulfide in the colonic luminal content is believed to contribute to inflammation and/or a risk of relapse of ulcerative colitis (33).

The presence of SQR places stringent constraints on this "sulfide signaling". One possibility may be that there is no signaling role for any endogenous circulating sulfide if "endogenous circulating sulfide" does not exist because of the presence of SQR, a point already discussed in (76) and (51), although this does not preclude the pharmacological use of sulfide. On the other hand, any of the following conditions would allow a physiologically relevant role for endogenous sulfide: a) physiological sulfide signaling occurs at very low concentrations (*e.g.*, below a threshold where SQR is not operating); b) sulfide is protected from the action of SQR; in this respect, the absence of SQR from the neural tissue is remarkable, and c) sulfide signaling occurs but mitochondrial SQR is the natural extinguisher of this signal; in this case, in tissues where SQR is expressed, sulfide signaling would occur within a very short time and over a short distance, as the probability of sulfide encountering SQR is very high.

Signaling is anticipation: Oxygen sensing

It may run counter to common sense that inhibitors of cytochrome oxidase could be used to improve tissue oxygenation by increasing blood flow. This apparent contradiction can be resolved if one considers that all these gaseous messengers (*i.e.*, NO, CO and sulfide) act as competitors for oxygen at the level of cytochrome oxidase (13). Thus, a cell exposed to NO, CO, or sulfide would functionally experience hypoxia at a higher oxygen concentration than in the absence of these gases. Consequently, if a cell displays a higher production rate of any of these gaseous messengers, it would be sensitized to hypoxic risk, triggering a response before the drop in oxygen consumption can harm neighboring cells. The possibility that sulfide is crucial to oxygen sensing has been studied extensively by Olson and his colleagues (50, 52–54). They demonstrated an association between sulfide exposure and sensitivity to oxygen in the carotid body. While the effect on the affinity of cytochrome oxidase for oxygen is shared by NO, CO, and sulfide, they exert contrasting effects on mitochondrial oxygen consumption: inhibition of cytochrome oxidase by NO and CO is expected to reduce the oxygen consumption of the cell. By contrast, in the presence of SQR, sulfide causes a rise in oxygen consumption well before sulfide can reach a concentration capable of inhibiting cytochrome oxidase. First, this can be expected to cause a self-amplification of the hypoxic signal and to potentiate NO, CO, or sulfide signaling. Second, sulfide will guarantee the safety of oxygen sensing cells against oxidative stress while NO or CO will not: indeed, the inhibitory action of NO on mitochondria tends to lead to a rise in the oxygen concentration (5) so that oxygen is made more available to form reactive oxygen species, including peroxynitrite that results from a direct reaction between NO and superoxide (65). Sulfide increases the oxygen consumption of mitochondria so that oxygen availability is decreased during hypoxic signaling, making the formation of reactive oxygen species less likely. Moreover, directly or not, sulfide is thought to be an opponent of oxidative stress (see above). Therefore, while NO or CO-based oxygen sensing would expose the oxygen-sensing cell to permanent oxidative stress, this will not be the case if oxygen sensing is based on the properties of sulfide.

The origin of eukaryotic cells and the compartmentalization of sulfide

The hypothesis concerning the formation of the primitive eukaryotic cell involves an association between an archaea and a bacterium. On the one hand, the archaea involved are related

to present-day species of thermophilic organisms, which produce sulfide because they use sulfur as the final electron acceptor. On the other hand, the bacterium at the origin of mitochondria had the potency to oxidize sulfide (58). In this model, the archaea would have been adapted to the continuous release of sulfide as metabolic waste and thus have been capable of tolerating relatively high sulfide concentrations, which in turn would protect it from oxygen toxicity. On the other hand, the sulfide user (bacterium) would have developed a high affinity for oxygen and sulfide in order to be able to use them before their levels became toxic. This model generates a eukaryotic cell that is a patchwork of structures of archaean origin adapted to high sulfide concentrations and those (of bacterial origin) that are not compatible with these high concentrations. This may explain why targets such as the K_{ATP} channel (believed to be involved in sulfide signaling) require such high sulfide concentrations (50–100 μM (30)), while mitochondria work constitutively to lower this concentration. It also suggests highly heterogeneous sulfide concentrations within the eukaryotic cell that could, in principle, account for its signaling properties and clearance. This situation may be comparable to the calcium signaling that is achieved via its compartmentalization in the cell (57). However, the gaseous nature of sulfide means that the concentration gradients would be extremely short-lived. An experimental demonstration of sulfide dynamics is then a challenge because it is necessary to establish that physiological changes in response to high sulfide concentrations can occur without poisoning mitochondria.

Experimental models with sulfide donors, sulfide, or ischemia?

Numerous studies have reported the beneficial effects of sulfide and the potential clinical interest of sulfide donors (64). Several of these experiments used NaHS or Na_2S as a source/form of sulfide (see Introduction). In fact, many experiments have used concentrations well above the 10–40 μM, the limit of toxicity reviewed in (64). Therefore, in experiments in which sulfide was added to a cell preparation or injected into an experimental animal model, it can be concluded that immediately after this addition, and proximal to the injection site, cells experienced inhibition of mitochondrial respiration. Thus, with the exception of oxidative stress, exposure to excessively high sulfide concentrations has the same consequences as ischemia. The dilution or destruction of sulfide gradually results in the restoration of steady-state sulfide concentrations without the oxidative stress associated with reperfusion. The intensity and duration of these sulfide effects are dependent on the ratio between the sulfide load and the metabolic capacity of the experimental system. This leads to the conclusion that in all experiments where cells were exposed to a sulfide concentration >40 μM, it caused a transient ischemia-reperfusion like period with reduced oxidative stress, inducing hypoxic signaling that eventually propagated to the rest of the organism. This is reminiscent of preconditioning effects during which a short period of ischemia, which does not cause significant damage, triggers adaptive changes that increase resistance to further ischemic shocks. It would therefore be difficult, and perhaps impossible, to separate effects solely attributable to sulfide from general bioenergetic effects.

Sulfide and Colonic Cells

Adaptation of colonocytes to sulfide exposure

Because sulfide is a potentially deleterious compound for the colonic epithelial cells when present at high levels, it is very important to identify the enzyme systems responsible for sulfide detoxification. Highly active thiol S-methyltransferase has been reported in the rat cecal and colonic mucosa (73). This enzyme catalyzes the methylation of H_2S in the presence of S-adenosylmethionine. However, based on more recent studies, it appears that the conversion of sulfide to thiosulfate represents the major process for sulfide detoxification in colonic epithelial cells. Thus, an analysis of cecal venous blood following the intracecal injection of radioactive H_2S in rats revealed that virtually all the H_2S absorbed was oxidized to thiosulfate (40). This high metabolic capacity appears to be a rather specialized function of the large intestine mucosa, because the other tissues tested were much less efficient (19). Rhodanese, which is expressed in the submucosa and crypts of the colon, was postulated to catalyze the reaction between sulfide and cyanide that forms thiocyanate (55). It now appears that rhodanese is not involved in the first step of H_2S metabolism, but more probably in a distal step of sulfide elimination (77). Both (27) and the stoichiometry observed during our study support the hypothesis of a "sulfide oxidizing unit" made up of three enzymatic activities (Fig. 1).

Another pertinent question concerns the adaptive capacity of colonic epithelial cells that have been incubated in the presence of high H_2S concentrations. During an *in vitro* study, it was shown that pretreatment of the human colonic epithelial cell line, HT-29, with 1 mM NaHS for 24 h induced neither cell necrosis nor apoptosis but caused a marked decrease in cell proliferation (39). Furthermore, NaHS pretreatment provoked a $>$4-fold increase in the capacity of HT-29 cells to produce lactate via anaerobic glycolysis. Because ATP, ADP, and AMP have been found to be unchanged by sulfide treatment despite the inhibitory effect of NaHS on oxygen consumption (39), it has been proposed that reduced cell division (and hence reduced energy consumption (10)) and increased glycolysis (and hence increased anaerobic energy production), constitute the adaptive response of HT-29 to high sulfide concentrations. It remains to be determined whether these conclusions from an *in vitro* experiment are relevant to the *in vivo* situation in the colonic lumen.

Reversion of mitochondrial complexes

While the first study from our laboratory using permeabilized cells (25) demonstrated a considerable difference in the sulfide oxidation rates of colonocytes and other cells, we subsequently showed that several primary cultures and transformed cell lines could rapidly oxidize sulfide (37). Indeed, a cell line derived from human monocytes was shown to approach the maximal theoretical sulfide consumption rate (37). In the same study, it was demonstrated that colonocytes displayed a qualitative difference that could greatly enhance their sulfide tolerance: when the oxidation of sulfide and carbon-derived substrates occurred simultaneously, electrons entered the respiratory chain via different pathways. Electrons from NADH (via complex I), $FADH_2$, succinate (complex II), and sulfide (SQR) converged on the Q pool that is re-oxidized by complex III (Fig. 1). Therefore, these electron

donors compete for the same electron acceptor. While at low sulfide concentrations the electron transfer from SQR shows precedence over complex I, this is not the case at high sulfide concentrations; in this situation, the addition of rotenone (inhibitor of complex I) increases the rate of sulfide oxidation in CHO cells (Fig. 2) or human monocytes (37). By contrast, with colonocytes, rotenone reduces the maximal rate of sulfide oxidation (37). Apparently, when sulfide exposure is high in colonocytes, complex I operates in a reverse mode and accepts electrons from quinone to reduce NAD to NADH (Fig. 3). This unfavorable redox reaction is balanced by a reversion of proton transfer in complex I and protons re-enter the mitochondrial matrix. Consequently, sulfide oxidation can occur even if the sulfide concentration is sufficient to inhibit cytochrome oxidase (Fig. 3). This unique adaptation of colonic epithelial cell mitochondria might be essential if they are to survive free sulfide concentrations of 60 μM. Similarly, the infusion of sulfide at a high rate results in an inhibition of respiration accompanied by a reversion of complex II (succinate dehydrogenase) in colonocytes, (Fig. 3) (25). Clearly, the recruitment of these reverse enzymatic processes is deleterious to mitochondrial bioenergetics that rely on the proton gradient to drive ATP synthesis (Fig. 1). To balance this negative effect on mitochondrial energy generation, two solutions can be envisaged: 1) a shift towards glycolytic energy production associated with export of the "redox debt" to other cells, or 2) intracellular/mitochondrial zoning, presented as a hypothesis to be tested in future studies (see below).

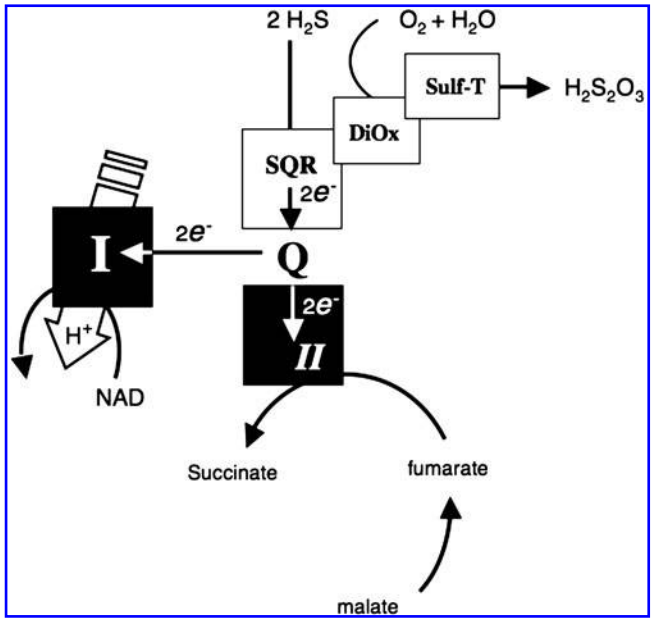


FIG. 3. Reversion of respiratory complexes by SQR. SQR can oxidize sulfide and reduce coenzyme Q, while coenzyme Q is re-oxidized by electron transfer to complexes II or I; in the latter case, electron transfer is accompanied by reverse proton transfer (compare with Fig. 1). In this scheme, complexes III and IV are not required for sulfide oxidation and are not included. They may be inactive if excess sulfide inhibits complex IV; in this case, the detoxification of sulfide continues slowly. Under such conditions, sulfide oxidation occurs at the expense of energy loss (*i.e.*, an influx of protons without the formation of ATP and the accumulation of a redox debt).

SQR as a Pharmacological Target

Mitochondrial poisoning may need to be prevented during a therapy-induced rise in the sulfide concentration, although the SQR present in many cells will work to rapidly lower this concentration. Thus, sulfide signaling needs to occur within a narrow concentration and time window. Because SQR can negate the effects of sulfide donors, it is itself a potential pharmacological target. In addition to using sulfide donors, the inhibition of SQR might constitute a second strategy to enhance sulfide-based signaling by raising the level of endogenous sulfide. The possible amplification of a natural signal by this mechanism has advantages over administering sulfide donors that increases sulfide concentrations systemically. While this might also be the case with SQR inhibitors, it is tempting to speculate that the neural tissue would be spared since it relies on mechanisms other than SQR for sulfide clearance, such as dilution and/or transport to a different location for oxidation. On the other hand, a potential adverse effect of inhibiting SQR might be that it impairs the ability of the colonic epithelium to protect itself against toxic concentrations of this molecule. The ideal strategy might then be to use competitive inhibitors as their effect would decline as sulfide concentration rises, thus protecting colonocytes.

Future Studies and Hypothesis

Bioenergetics and signaling

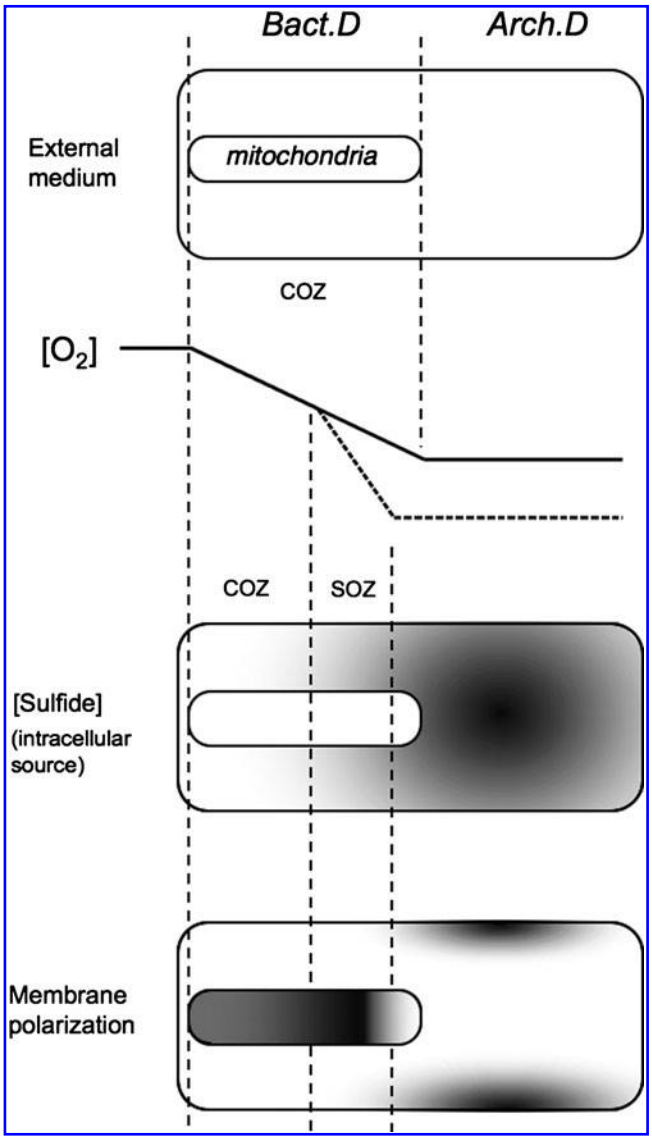
Methods that estimate free rather than total sulfide (18, 68, 76) and the characteristics of mammalian mitochondrial SQR (25, 37) argue in favor of extremely low concentrations of free sulfide in the extracellular compartment. However, even low sulfide concentrations (20–30 nM) have been shown to trigger signaling (47). Therefore, if a threshold exists for the activity of SQR in the 20 nM range, it is possible that sulfide signaling may occur independently of mitochondrial bioenergetics. The high concentrations used in pharmacological experiments (64) would serve to compensate for the instability of sulfide and/or promote adaptive mechanisms distinct from sulfide signaling (see above). However, during such studies, it is still necessary to make a distinction between "true sulfide signaling" that continues to occur at nanomolar concentrations and the transient bioenergetic influences that are dependent on micromolar concentrations.

Sulfide gradients

Our data have shown that a sulfide sink exists in most cells: the mitochondrial SQR. What remains a matter of debate is the potency of sulfide sources within cells, for example, see (41). All the data reporting a relatively high sulfide content in tissues could either be considered to be artifacts or may result from a relatively high concentration of sulfide sources that remain to be characterized. There are several observations that support the importance of the biological effects mediated at high sulfide concentrations (100 μM) These include: a) the potential release of sulfide from bound sulfane sulfur stores (29, 35), b) the opposing effects of inhibitors of sulfide-producing enzymes and high concentrations of sulfide on the same physiological parameter (17), and c) the direct effect of sulfide on the K_{ATP} channel (30). Therefore, if sulfide sources exist that can locally increase the concentration of sulfide to micromolar values, sulfide gradients would be determined

by the location of a sulfide-producing source relative to the mitochondrial sulfide sink. This is likely to occur within the same cell. Figure 4 illustrates the consequences of such proximity in terms of oxygen availability, mitochondrial metabolism, and membrane potentials. Furthermore, such gradients may also exist within the mitochondrion itself if 3MST is indeed a source of H_2S. The loss of expression of SQR in neural tissue could be interpreted as an adaptation to enhance sulfide stability so that its action can be exerted. Demonstration of the existence of these sulfide sources, and of the sulfide gradient, is a challenge for the future, and would strongly support a physiologically relevant role for sulfide.

One example of a cell type in which a sulfide gradient is expected to exist is the colonocyte, although in this situation the sulfide also derives from external sources (Fig. 5). At the apical end of the cell, the villi are in contact with a sulfide-rich medium. Mitochondria just beneath the villi are therefore exposed to sulfide concentrations that are high enough to inhibit cytochrome oxidase. However, sulfide oxidation may proceed through reverse electron transfer (37) (Fig. 3).



Within living cells, mitochondria are now considered to constitute an extended filamentous network (49) rather than the ovoid organelles resulting from a differential centrifugation of homogenates, or as suggested by electron microscopy images obtained on thin sections of cells. Therefore, if at the apical end of the network SQR is supplied with NAD, it may neutralize sulfide while at the other end of the same network, normal bioenergetics re-oxidize the NADH generating the membrane potential and driving ATP synthesis. A plausible consequence of this regionalization within mitochondria could be a different localization of cytochrome oxidase and SQR, because it would be futile to load the apical end with cytochrome oxidase. In fact, the exposure of (non-polarized) colonocytes to sulfide has been shown to lead to a reduction in cytochrome oxidase expression (39). This hypothesis, that different domains of the mitochondrial network have different functions based on their localization in the apical versus basolateral ends of the colonocyte, needs to be tested experimentally.

Sulfide and mitochondrial dynamics

Mitochondria undergo fusion and fission, processes that occur at a relatively high rate (49). The depolarization of mitochondria leads to fragmentation, probably because fusion requires more energy (38). The occurrence of a sulfide source close to mitochondria would create a sulfide concentration gradient. Near to the source, the mitochondrial bioenergetics would be compromised by sulfide poisoning and thus mitochondria would be prone to fission and destabilization (48).

FIG. 4. Heterogeneous sulfide concentrations within a single cell. The eukaryotic cell (*large rectangle*) is schematized based on the hypothesis of the juxtaposition of a bacterial domain (Bact. D) and an archaeal domain (Arch. D). The bacterial domain contains mitochondria and is oriented towards the oxygen-containing external medium, situated exclusively on the *left* in this scheme. *Top:* the bacterial domain consumes oxygen to oxidize carbon-containing substrates (COZ, carbon oxidizing zone), then the oxygen concentration declines gradually from the outside to the inside in the presence of mitochondria ([O_2] *solid line*). *Middle:* if the archaeal domain releases sulfide, the mitochondria oxidize it, creating a sulfide-oxidizing zone (SOZ). This sulfide oxidation consumes more oxygen (three times more if maximal). The drop in the oxygen concentration is steeper in the SOZ, leading to a significantly lower oxygen concentration in the internal (Archaeal) domain of the cell ([O_2] *dotted line*). *Bottom:* the effects of sulfide and oxygen gradients on membrane potential. If sulfide release is high enough, then the mitochondrial side close to the sulfide-producing source will be subject to poisoning of complex IV. This will prevent the extension of the bacterial domain into the archaeal domain, because mitochondria with a compromised bioenergetics, here shown as a locally depolarized zone, are unstable. Beyond this zone, sulfide concentrations will allow oxidation without poisoning the respiratory chain (a range of roughly 20–200 n*M*). At the level of the plasma membrane, sulfide causes increased conductance through the K_{ATP} channel, resulting in hyperpolarization. In this scheme, while sulfide may locally reach significant concentrations within a cell, it will not be released at concentrations above the SQR threshold in the external medium.

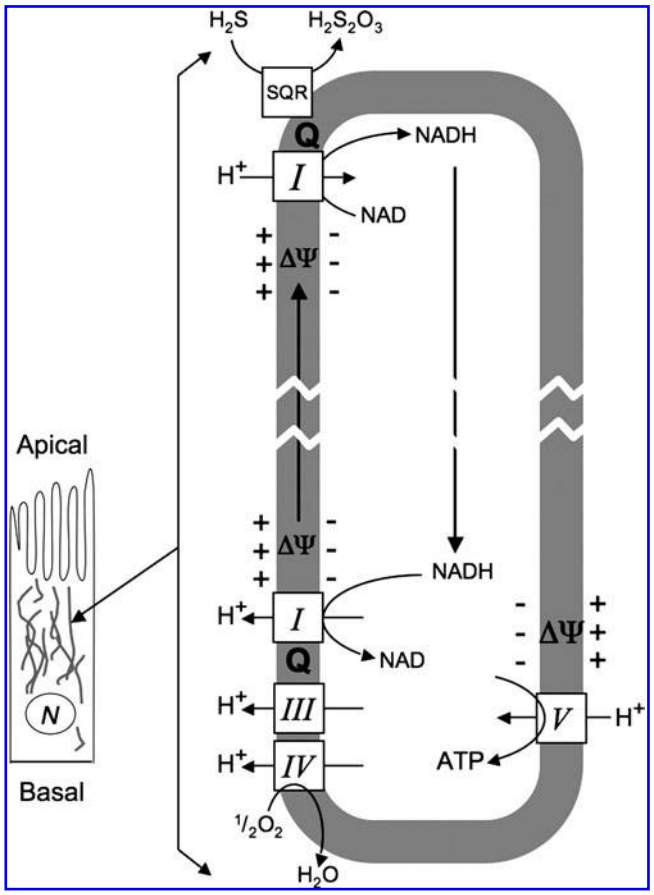


FIG. 5. Collaboration between the apical and basal domains in colonocyte mitochondria. *Left:* Scheme of a colonocyte, which is a column-shaped cell with microvilli at the apical end and the opposite basal side (N, nucleus). The mitochondria form a network (*gray*) that is located between these two opposite sides of the cell. *Right:* Close-up of a mitochondrion with its apical and basal sides at the *top* and *bottom*, respectively. The mitochondrial respiratory complexes in the membrane (*thick gray line*) are shown as numbered boxes (I–V). SQR is shown at the apical end of the mitochondrion that is exposed to a high concentration of sulfide and can oxidize it at a maximal rate through reverse electron transfer to complex I (see Fig. 3), releasing NADH. On the basal side, the respiratory complexes operate in a conventional mode and oxidize NADH to generate a proton gradient and a membrane potential that can be used to drive ATP synthesis (*bottom right*). This cooperation requires an input of redox equivalents (NADH, *downwards arrow* in the mitochondrial matrix). Reverse electron transfer will be helped if the membrane potential generated at the basal end propagates towards the apical end (delocalized bioenergetics, *upwards arrow* in the membrane).

At a greater distance, SQR activity may polarize mitochondria and also limit sulfide diffusion. According to this theory, the sulfide dynamics suggested above might be reflected by, or be a causative factor of, mitochondrial dynamics. As a preliminary step, it would be extremely interesting to determine the effect of sulfide production inhibitors on mitochondrial dynamics. Of interest is the fact that these hypotheses are pertinent to the debate concerning delocalized versus localized bioenergetics in the mitochondrial network (compare Figs. 4 and 5).

Acknowledgments

This work was supported by the Institut National de la Recherche Agronomique (INRA) and AgroParisTech, the Centre National de la Recherche Scientifique (CNRS), and the Institut de la Santé et Recherche Médicale (INSERM). The authors wish to thank Vicky Hawken for editing our manuscript.

References

1. Abe K and Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J Neurosci* 16: 1066–1071, 1996.
2. Baglieri A, Mahe S, Zidi S, Huneau JF, Thuillier F, Marteau P, and Tome D. Gastro-jejunal digestion of soya-bean-milk protein in humans. *Br J Nutr* 72: 519–532, 1994.
3. Bailly X and Vinogradov S. The sulfide binding function of annelid hemoglobins: Relic of an old biosystem? *J Inorg Biochem* 99: 142–150, 2005.
4. Bartholomew TC, Powell GM, Dodgson KS, and Curtis CG. Oxidation of sodium sulphide by rat liver, lungs and kidney. *Biochem Pharmacol* 29: 2431–2437, 1980.
5. Benamar A, Rolletschek H, Borisjuk L, Avelange–Macherel MH, Curien G, Mostefai HA, Andriantsitohaina R, and Macherel D. Nitrite–nitric oxide control of mitochondrial respiration at the frontier of anoxia. *Biochim Biophys Acta* 1777: 1268–1275, 2008.
6. Benavides GA, Squadrito GL, Mills RW, Patel HD, Isbell TS, Patel RP, Darley–Usmar VM, Doeller JE, and Kraus DW. Hydrogen sulfide mediates the vasoactivity of garlic. *Proc Natl Acad Sci USA* 104: 17977–17982, 2007.
7. Blachier F, Davila AM, Mimoun S, Benetti PH, Atanasiu C, Andriamihaja M, Benamouzig R, Bouillaud F, and Tome D. Luminal sulfide and large intestine mucosa: Friend or foe? *Amino Acids* 39: 335–347, 2009.
8. Blachier F, Mariotti F, Huneau JF, and Tome D. Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. *Amino Acids* 33: 547–562, 2007.
9. Bos C, Juillet B, Fouillet H, Turlan L, Dare S, Luengo C, N'Tounda R, Benamouzig R, Gausseres N, Tome D, and Gaudichon C. Postprandial metabolic utilization of wheat protein in humans. *Am J Clin Nutr* 81: 87–94, 2005.
10. Buttgereit F and Brand MD. A hierarchy of ATP-consuming processes in mammalian cells. *Biochem J* 312: 163–167, 1995.
11. Chacko A and Cummings JH. Nitrogen losses from the human small bowel: Obligatory losses and the effect of physical form of food. *Gut* 29: 809–815, 1988.
12. Chiku T, Padovani D, Zhu W, Singh S, Vitvitsky V, and Banerjee R. H2S 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: 11601–11612, 2009.
13. Cooper CE and Brown GC. The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: Chemical mechanism and physiological significance. *J Bioenerg Biomembr* 40: 533–539, 2008.
14. Evenepoel P, Claus D, Geypens B, Hiele M, Geboes K, Rutgeerts P, and Ghoos Y. Amount and fate of egg protein

escaping assimilation in the small intestine of humans. *Am J Physiol* 277: G935–943, 1999.
15. Fiorucci S, Distrutti E, Cirino G, and Wallace JL. The emerging roles of hydrogen sulfide in the gastrointestinal tract and liver. *Gastroenterology* 131: 259–271, 2006.
16. Florin T, Neale G, Gibson GR, Christl SU, and Cummings JH. Metabolism of dietary sulphate: Absorption and excretion in humans. *Gut* 32: 766–773, 1991.
17. Fu Z, Liu X, Geng B, Fang L, and Tang C. Hydrogen sulfide protects rat lung from ischemia-reperfusion injury. *Life Sci* 82: 1196–1202, 2008.
18. Furne J, Saeed A, and Levitt MD. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. *Am J Physiol Regul Integr Comp Physiol* 295: R1479–1485, 2008.
19. Furne J, Springfield J, Koenig T, DeMaster E, and Levitt MD. Oxidation of hydrogen sulfide and methanethiol to thiosulfate by rat tissues: A specialized function of the colonic mucosa. *Biochem Pharmacol* 62: 255–259, 2001.
20. Gaill F. Aspects of life development at deep sea hydrothermal vents. *FASEB J* 7: 558–565, 1993.
21. Gaudichon C, Bos C, Morens C, Petzke KJ, Mariotti F, Everwand J, Benamouzig R, Dare S, Tome D, and Metges CC. Ileal losses of nitrogen and amino acids in humans and their importance to the assessment of amino acid requirements. *Gastroenterology* 123: 50–59, 2002.
22. Gausseres N, Mahe S, Benamouzig R, Luengo C, Drouet H, Rautureau J, and Tome D. The gastro-ileal digestion of 15N-labelled pea nitrogen in adult humans. *Br J Nutr* 76: 75–85, 1996.
23. Gibson GR, Cummings JH, and Macfarlane GT. Competition for hydrogen between sulphate-reducing bacteria and methanogenic bacteria from the human large intestine. *J Appl Bacteriol* 65: 241–247, 1988.
24. Gibson GR, Cummings JH, and Macfarlane GT. Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. *Appl Environ Microbiol* 54: 2750–2755, 1988.
25. Goubern M, Andriamihaja M, Nubel T, Blachier F, and Bouillaud F. Sulfide, the first inorganic substrate for human cells. *FASEB J* 21: 1699–1706, 2007.
26. Grieshaber MK and Volkel S. Animal adaptations for tolerance and exploitation of poisonous sulfide. *Annu Rev Physiol* 60: 33–53, 1998.
27. Hildebrandt TM and Grieshaber MK. Three enzymatic activities catalyze the oxidation of sulfide to thiosulfate in mammalian and invertebrate mitochondria. *FEBS J* 275: 3352–3361, 2008.
28. Hoerter J, Gonzalez–Barroso MD, Couplan E, Mateo P, Gelly C, Cassard–Doulcier AM, Diolez P, and Bouillaud F. Mitochondrial uncoupling protein 1 expressed in the heart of transgenic mice protects against ischemic-reperfusion damage. *Circulation* 110: 528–533, 2004.
29. Ishigami M, Hiraki K, Umemura K, Ogasawara Y, Ishii K, and Kimura H. A source of hydrogen sulfide and a mechanism of its release in the brain. *Antioxid Redox Signal* 11: 205–214, 2009.
30. Jiang B, Tang G, Cao K, Wu L, and Wang R. Molecular mechanism for H(2)S-induced activation of K(ATP) channels. *Antioxid Redox Signal* 12: 1167–1178, 2010.
31. Johnson KS, Beehler CL, Sakamoto–Arnold CM, and Childress JJ. In situ measurements of chemical distributions in a deep-sea hydrothermal vent field. *Science* 231: 1139–1141, 1986.
32. Jorgensen J and Mortensen PB. Hydrogen sulfide and colonic epithelial metabolism: Implications for ulcerative colitis. *Dig Dis Sci* 46: 1722–1732, 2001.
33. Jowett SL, Seal CJ, Pearce MS, Phillips E, Gregory W, Barton JR, and Welfare MR. Influence of dietary factors on the clinical course of ulcerative colitis: A prospective cohort study. *Gut* 53: 1479–1484, 2004.
34. Kabil O and Banerjee R. The redox biochemistry of hydrogen sulfide. *J Biol Chem* 285: 21903–21907, 2010.
35. Kimura H. Hydrogen sulfide: Its production, release and functions. *Amino Acids* 2010 [Epub ahead of print]; DOI: 10.1007/s00726-010-0510-x.
36. Kimura Y, Goto Y, and Kimura H. Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria. *Antioxid Redox Signal* 12: 1–13, 2010.
37. Lagoutte E, Mimoun S, Andriamihaja M, Chaumontet C, Blachier F, and Bouillaud F. Oxidation of hydrogen sulfide remains a priority in mammalian cells and causes reverse electron transfer in colonocytes. *Biochim Biophys Acta* 1797: 1500–1511, 2010.
38. Legros F, Lombes A, Frachon P, and Rojo M. Mitochondrial fusion in human cells is efficient, requires the inner membrane potential, and is mediated by mitofusins. *Mol Biol Cell* 13: 4343–4354, 2002.
39. Leschelle X, Goubern M, Andriamihaja M, Blottiere HM, Couplan E, Gonzalez–Barroso MD, Petit C, Pagniez A, Chaumontet C, Mignotte B, Bouillaud F, and Blachier F. Adaptative metabolic response of human colonic epithelial cells to the adverse effects of the luminal compound sulfide. *Biochim Biophys Acta* 1725: 201–12, 2005.
40. Levitt MD, Furne J, Springfield J, Suarez F, and DeMaster E. Detoxification of hydrogen sulfide and methanethiol in the cecal mucosa. *J Clin Invest* 104: 1107–1114, 1999.
41. Linden DR, Sha L, Mazzone A, Stoltz GJ, Bernard CE, Furne JK, Levitt MD, Farrugia G, and Szurszewski JH. Production of the gaseous signal molecule hydrogen sulfide in mouse tissues. *J Neurochem* 106: 1577–1585, 2008.
42. Macfarlane G and Cummings J. The colonic flora, fermentation and large bowel digestive function. In: *The Large Intestine: Physiology, Pathophysiology and Disease*. Philipps S, Pemberton J, Shorter R.eds. New York: Raven Press; 1991. pp. 51–92.
43. Macfarlane GT, Gibson GR, and Cummings JH. Comparison of fermentation reactions in different regions of the human colon. *J Appl Bacteriol* 72: 57–64, 1992.
44. Magee EA, Richardson CJ, Hughes R, and Cummings JH. Contribution of dietary protein to sulfide production in the large intestine: An *in vitro* and a controlled feeding study in humans. *Am J Clin Nutr* 72: 1488–1494, 2000.
45. Mathai JC, Missner A, Kugler P, Saparov SM, Zeidel ML, Lee JK, and Pohl P. No facilitator required for membrane transport of hydrogen sulfide. *Proc Natl Acad Sci USA* 106: 16633–16638, 2009.
46. Mitsui T, Edmond LM, Magee EA, and Cummings JH. The effects of bismuth, iron, zinc and nitrate on free sulfide in batch cultures seeded with fecal flora. *Clin Chim Acta* 335: 131–135, 2003.
47. Muzaffar S, Shukla N, Bond M, Newby AC, Angelini GD, Sparatore A, Del Soldato P, and Jeremy JY. Exogenous hydrogen sulfide inhibits superoxide formation, NOX-1 expression and Rac1 activity in human vascular smooth muscle cells. *J Vasc Res* 45: 521–528, 2008.
48. Narendra D, Tanaka A, Suen DF, and Youle RJ. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J Cell Biol* 183: 795–803, 2008.

49. Okamoto K and Shaw JM. Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. *Annu Rev Genet* 39: 503–536, 2005.
50. Olson KR. Vascular actions of hydrogen sulfide in nonmammalian vertebrates. *Antioxid Redox Signal* 7: 804–812, 2005.
51. Olson KR. Is hydrogen sulfide a circulating "gasotransmitter" in vertebrate blood? *Biochim Biophys Acta* 1787: 856–863, 2009.
52. Olson KR, Dombkowski RA, Russell MJ, Doellman MM, Head SK, Whitfield NL, and Madden JA. Hydrogen sulfide as an oxygen sensor/transducer in vertebrate hypoxic vasoconstriction and hypoxic vasodilation. *J Exp Biol* 209: 4011–4023, 2006.
53. Olson KR, Forgan LG, Dombkowski RA, and Forster ME. Oxygen dependency of hydrogen sulfide-mediated vasoconstriction in cyclostome aortas. *J Exp Biol* 211: 2205–2213, 2008.
54. Olson KR and Whitfield NL. Hydrogen sulfide and oxygen sensing in the cardiovascular system. *Antioxid Redox Signal* 12: 1219–1234, 2010.
55. Picton R, Eggo MC, Merrill GA, Langman MJ, and Singh S. Mucosal protection against sulphide: Importance of the enzyme rhodanese. *Gut* 50: 201–205, 2002.
56. Reiffenstein RJ, Hulbert WC, and Roth SH. Toxicology of hydrogen sulfide. *Annu Rev Pharmacol Toxicol* 32: 109–134, 1992.
57. Rizzuto R, Pinton P, Brini M, Chiesa A, Filippin L, and Pozzan T. Mitochondria as biosensors of calcium microdomains. *Cell Calcium* 26: 193–199, 1999.
58. Searcy DG. Metabolic integration during the evolutionary origin of mitochondria. *Cell Res* 13: 229–238, 2003.
59. Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, Ishii K, and Kimura H. 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid Redox Signal* 11: 703–714, 2009.
60. Silvester KR and Cummings JH. Does digestibility of meat protein help explain large bowel cancer risk? *Nutr Cancer* 24: 279–288, 1995.
61. Singh S, Padovani D, Leslie RA, Chiku T, and Banerjee R. Relative contributions of cystathionine beta-synthase and gamma-cystathionase to H2S biogenesis via alternative transsulfuration reactions. *J Biol Chem* 284: 22457–22466, 2009.
62. Stipanuk MH. Sulfur amino acid metabolism: Pathways for production and removal of homocysteine and cysteine. *Annu Rev Nutr* 24: 539–577, 2004.
63. Suarez F, Furne J, Springfield J, and Levitt M. Production and elimination of sulfur-containing gases in the rat colon. *Am J Physiol* 274: G727–733, 1998.
64. Szabo C. Hydrogen sulphide and its therapeutic potential. *Nat Rev Drug Discov* 6: 917–935, 2007.
65. Szabo C, Ischiropoulos H, and Radi R. Peroxynitrite: Biochemistry, pathophysiology and development of therapeutics. *Nat Rev Drug Discov* 6: 662–680, 2007.
66. Tiranti V, Viscomi C, Hildebrandt T, Di Meo I, Mineri R, Tiveron C, Levitt MD, Prelle A, Fagiolari G, Rimoldi M, and Zeviani M. Loss of ETHE1, a mitochondrial dioxygenase, causes fatal sulfide toxicity in ethylmalonic encephalopathy. *Nat Med* 15: 200–205, 2009.
67. Turrens JF. Superoxide production by the mitochondrial respiratory chain. *Biosci Rep* 17: 3–8, 1997.
68. Ubuka T. Assay methods and biological roles of labile sulfur in animal tissues. *J Chromatogr B Analyt Technol Biomed Life Sci* 781: 227–249, 2002.
69. Ubuka T, Abe T, Kajikawa R, and Morino K. Determination of hydrogen sulfide and acid-labile sulfur in animal tissues by gas chromatography and ion chromatography. *J Chromatogr B Biomed Sci Appl* 757: 31–37, 2001.
70. Volkel S and Grieshaber M. Sulphide oxidation and oxidative phosphorylation in the mitochondria of the lugworm. *J Exp Biol* 200: 83–92, 1997.
71. Wang R. Two's company, three's a crowd: Can H2S be the third endogenous gaseous transmitter? *FASEB J* 16: 1792–1798, 2002.
72. Wang R. The gasotransmitter role of hydrogen sulfide. *Antioxid Redox Signal* 5: 493–501, 2003.
73. Weisiger RA, Pinkus LM, and Jakoby WB. Thiol S-methyltransferase: Suggested role in detoxication of intestinal hydrogen sulfide. *Biochem Pharmacol* 29: 2885–2887, 1980.
74. Whiteman M, Armstrong JS, Chu SH, Jia-Ling S, Wong BS, Cheung NS, Halliwell B, and Moore PK. The novel neuromodulator hydrogen sulfide: An endogenous peroxynitrite 'scavenger'? *J Neurochem* 90: 765–768, 2004.
75. Whiteman M, Cheung NS, Zhu YZ, Chu SH, Siau JL, Wong BS, Armstrong JS, and Moore PK. Hydrogen sulphide: A novel inhibitor of hypochlorous acid-mediated oxidative damage in the brain? *Biochem Biophys Res Commun* 326: 794–798, 2005.
76. Whitfield NL, Kreimier EL, Verdial FC, Skovgaard N, and Olson KR. Reappraisal of H2S/sulfide concentration in vertebrate blood and its potential significance in ischemic preconditioning and vascular signaling. *Am J Physiol Regul Integr Comp Physiol* 294: R1930–1937, 2008.
77. Wilson K, Mudra M, Furne J, and Levitt M. Differentiation of the roles of sulfide oxidase and rhodanese in the detoxification of sulfide by the colonic mucosa. *Dig Dis Sci* 53: 277–283, 2008.
78. Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, and Wang R. H2S as a physiologic vasorelaxant: Hypertension in mice with deletion of cystathionine gamma-lyase. *Science* 322: 587–590, 2008.
79. Yong R and Searcy DG. Sulfide oxidation coupled to ATP synthesis in chicken liver mitochondria. *Comp Biochem Physiol B Biochem Mol Biol* 129: 129–137, 2001.
80. Zhao W, Zhang J, Lu Y, and Wang R. The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. *EMBO J* 20: 6008–6016, 2001.

Address correspondence to:
Dr. Frédéric Bouillaud
Institut Cochin
Université Paris Descartes
INSERM-U1016, CNRS-UMR8104
24 rue du Faubourg St. Jacques
75014 Paris
France

E-mail: frederic.bouillaud@inserm.fr

Date of first submission to ARS Central, October 4, 2010; date of acceptance, October 28, 2010.

Abbreviations Used

3MST = 3-mercaptopyruvate sulfur transferase
CBS = cystathionine ß-synthase
CSE = cystathionine γ-lyase
GSH = reduced glutathione
ROS = reactive oxygen species
SQR = sulfide quinone reductase

This article has been cited by:

1. Jungwoo Shin, Seon-Jin Choi, Doo-Young Youn, Il-Doo Kim. 2012. Exhaled VOCs sensing properties of WO3 nanofibers functionalized by Pt and IrO2 nanoparticles for diagnosis of diabetes and halitosis. *Journal of Electroceramics* **29**:2, 106-116. [CrossRef]
2. Jose Matallo, Josef Vogt, Michael Georgieff, Csaba Szabó, Peter Radermacher, Enrico Calzia. 2012. P31 Sulfide metabolism at low oxygen conditions. *Nitric Oxide* **27**, S25-S26. [CrossRef]
3. Mikko Nikinmaa. 2012. Climate change and ocean acidification–interactions with aquatic toxicology. *Aquatic Toxicology* . [CrossRef]
4. Sabria Mimoun , Mireille Andriamihaja , Catherine Chaumontet , Calina Atanasiu , Robert Benamouzig , Jean Marc Blouin , Daniel Tomé , Frédéric Bouillaud , François Blachier . 2012. Detoxification of H2S by Differentiated Colonic Epithelial Cells: Implication of the Sulfide Oxidizing Unit and of the Cell Respiratory Capacity. *Antioxidants & Redox Signaling* **17**:1, 1-10. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
5. Victor Vitvitsky , Omer Kabil , Ruma Banerjee . 2012. High Turnover Rates for Hydrogen Sulfide Allow for Rapid Regulation of Its Tissue Concentrations. *Antioxidants & Redox Signaling* **17**:1, 22-31. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
6. Jerzy Be#towski , Anna Jamroz-Wi#niewska . 2012. Modulation of H2S Metabolism by Statins: A New Aspect of Cardiovascular Pharmacology. *Antioxidants & Redox Signaling* **17**:1, 81-94. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. Philippe Haouzi. 2012. Ventilatory and metabolic effects of exogenous hydrogen sulfide. *Respiratory Physiology & Neurobiology* . [CrossRef]
8. Kenneth R. Olson. 2012. Mitochondrial adaptations to utilize hydrogen sulfide for energy and signaling. *Journal of Comparative Physiology B* . [CrossRef]
9. Mayumi Kajimura, Tsuyoshi Nakanishi, Toshiki Takenouchi, Takayuki Morikawa, Takako Hishiki, Yoshinori Yukutake, Makoto Suematsu. 2012. Gas biology: Tiny molecules controlling metabolic systems. *Respiratory Physiology & Neurobiology* . [CrossRef]
10. Eric R. DeLeon, Gilbrian F. Stoy, Kenneth R. Olson. 2012. Passive loss of hydrogen sulfide in biological experiments. *Analytical Biochemistry* **421**:1, 203-207. [CrossRef]
11. Kenneth R. Olson. 2011. Hydrogen sulfide is an oxygen sensor in the carotid body. *Respiratory Physiology & Neurobiology* . [CrossRef]
12. Ruma Banerjee . 2011. Hydrogen Sulfide: Redox Metabolism and Signaling. *Antioxidants & Redox Signaling* **15**:2, 339-341. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
13. Omer Kabil , Victor Vitvitsky , Peter Xie , Ruma Banerjee . 2011. The Quantitative Significance of the Transsulfuration Enzymes for H2S Production in Murine Tissues. *Antioxidants & Redox Signaling* **15**:2, 363-372. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]