Cysteine-derived hydrogen sulfide and gut health: a matter of endogenous or bacterial origin

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\textbf{Purpose of review} 
Hydrogen sulfide (H\textsubscript{2}S) is produced in the gut from cysteine by epithelial cells and by the intestinal microbiota. Initially considered as a toxic gas, the pleiotropic effects of H\textsubscript{2}S are now recognized, especially in the colonic mucosa. The aim of this review is to present new experimental data indicating that cysteine-derived H\textsubscript{2}S is emerging as a key regulator of gut health.

\textbf{Recent findings} 
Cysteine degradation by the microbiota emerged as a dominant pathway for H\textsubscript{2}S production. Among bacteria producing H\textsubscript{2}S from cysteine, \textit{Fusobacterium} appears as a pivotal genus associated with digestive diseases. H\textsubscript{2}S promotes or alleviates mucosal inflammation, mostly according to its high (high micromolar to millimolar) or low (nanomolar to low micromolar) concentration, respectively. H\textsubscript{2}S maintains the integrity of the mucus layer when derived from endogenous metabolism but is detrimental for this parameter when produced in excess by gut microbes. In inflammatory bowel diseases, an upregulation of H\textsubscript{2}S production from cysteine by the gut microbiota is observed concomitantly with a downregulation of enzymes implicated in its mucosal detoxification. In colorectal cancer patients, an upregulation of both endogenous and microbial H\textsubscript{2}S production from cysteine are observed at tumor site that might contribute to disease progression.

\textbf{Summary} 
H\textsubscript{2}S is a double-edge sword for the intestinal epithelium. This is related to the bell-shaped effects of H\textsubscript{2}S, with protective effect at low concentration but deleterious effects at higher concentrations. As the gut microbiota produces much more H\textsubscript{2}S from cysteine than endogenous metabolism, we consider that the bacterial or epithelial source of H\textsubscript{2}S is a major determinant of its effects for intestinal health.

\textbf{Keywords} 
colorectal cancer, epithelium, H\textsubscript{2}S, intestinal inflammation, microbiota, sulfide quinone reductase

\textbf{INTRODUCTION} 
The colonic and rectal epithelium face a very complex mixture of solid and liquid matters, which contains microorganisms, residual dietary compounds, endogenous products, and bacterial metabolites. Although the large intestine is covered by mucus layers, it is well known that some of the compounds present in the luminal content can cross these layers and enter the colonic epithelial cells through the apical membranes \cite{1}. The mammalian colonic epithelium is characterized by a structure of crypts separated by the surface epithelium. This structure, which is one of the most rapid self-renewing entities in the body, is replaced within a few days thanks to asymmetric mitosis of the pluripotent leucine-rich repeat-containing G-protein-coupled receptor 5 stem cells, followed by differentiation in different cell lineages, and exfoliation of the fully mature epithelial cells in the colonic lumen.

The colonic epithelium, which is largely responsible for luminal water absorption and electrolyte absorption and secretion, must follow a well coordinated sequence of events to allow its homeostatic renewal and the maintenance of its selective barrier function. Indeed, the alteration of this latter...
function is considered as an event that likely favors the mucosal inflammatory process in predisposed individuals [2]. In addition, the reiteration of colonic or rectal inflammatory episodes is known to increase the long-term risk of colonic and rectal preneoplasia and neoplasia in the so-called colitis-associated cancer [3]. Regarding this latter point, it is worth noting that the colonic crypt stem cells appear to be at the origin of colorectal cancer (CRC) [4*].

The idea that some compounds originating from the metabolic activity of the intestinal microbiota toward undigested or not fully digested dietary compounds in the intestinal content may impact the metabolism and physiology of the colonic mucosa was already proposed by Macfarlane and Cummings more than two decades ago [5]. More recently, this important concept has received confirmation thanks to studies showing that modifications of the luminal concentrations of individual bacterial metabolites may impact positively or negatively the risk of colon/rectum inflammation or neoplasia. This has been notably the case regarding hydrogen sulfide (H$_2$S). Several studies revealed that modifications of the production of this fascinating gaseous compound by either the colonic bacteria or by the colonocytes are protective or deleterious toward the colonic epithelium in different physiological and pathological contexts.

Thus, the aim of the present review is to present recent data related to the H$_2$S synthesis from cysteine by the bacteria and by the colonic epithelial cells, and to evaluate to what extent such H$_2$S production is involved in normal colonic epithelium physiology or may intervene in the course of colonic inflammation and neoplasia.
high micromolar to low millimolar concentrations [12]. Overall, it appears that H$_2$S that is not bound to luminal compounds is the one that will diffuse through the mucous layers and then into the colonocytes. In addition, the characteristics of the diet may influence the amount of luminal sulfide in the large intestine, as an increased protein consumption in the rat model increases the amount of H$_2$S measured by gas chromatography–mass spectrometry in both the caecum and colon [14*].

In mammalian cells, although the respective roles of the different metabolic pathways for the synthesis of H$_2$S from cysteine in the different cell phenotypes are not clear yet, several operating metabolic pathways have been described [15,16*] (Fig. 1). Cystathionine β-synthase (CBS) appears to be a versatile enzyme as it can convert cysteine to H$_2$S and serine but can also convert two molecules of cysteine into H$_2$S and lanthionine. In addition, CBS can convert cysteine and homocysteine into H$_2$S and cystathionine. CBS is not the only enzyme involved in H$_2$S production from cysteine. Indeed, cystathionine γ-lyase (CSE) converts cysteine into H$_2$S, NH$_3$, and pyruvate, and cysteine and homocysteine into H$_2$S and cystathionine. Lastly, by the sequential activity of cysteine aminotransferase and 3-mercaptopyruvate sulfurtransferase (3-MST), cysteine can give rise to H$_2$S production.

In normal colonocytes, there is a paucity of information regarding the capacity of these cells for H$_2$S production and the metabolic pathways involved in such endogenous production. In the mammalian colon, CBS appears to be the major H$_2$S-synthesizing enzyme [9].

**Effects of exogenous and endogenous hydrogen sulfide and energy metabolism in colonocytes**

In colonocytes isolated from the rat colon, 20 and 40 μM NaHS, used as a rapid H$_2$S donor, increase instantaneously the cell oxygen consumption by entering the mitochondrial respiratory chain at the level of the sulfide quinone reductase (SQR) (Fig. 2) [14*]. This enzyme oxidized H$_2$S by transferring electron equivalents to quinones with the intervention of reduced glutathione, and then to the mitochondrial complexes III and IV (cytochrome c oxidase). In the meantime, the persulfide dioxygenase (ETHE1) allows the synthesis of sulfite, which is converted to thiosulfate by rhodanese, an enzyme characterized by its sulfur transferase activity [17*].

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**FIGURE 1.** Metabolic pathways allowing H$_2$S synthesis from cysteine in mammalian cells and synthesis of H$_2$S by the intestinal microbiota. This schematic representation recapitulates the metabolic pathways allowing H$_2$S biosynthesis in mammalian cells, notably presumably in colonic epithelial cells, although the main metabolic pathways used by colonocytes for H$_2$S synthesis from cysteine are not entirely elucidated. On the bottom of the figure is represented the metabolic pathway allowing H$_2$S synthesis in gut bacteria from cysteine. CAT, cysteine aminotransferase; CBS, cystathionine β-synthase; CD, cysteine desulphhydrase; CSE, cystathionine γ-lyase; H$_2$S, hydrogen sulfide; 3-MST, 3-mercaptopyruvate sulfurtransferase.
consumption by colonocytes is associated with an inner mitochondrial membrane energization and ATP synthesis. Thus, the detoxification of $\text{H}_2\text{S}$ by the sulfide-oxidizing unit allows energy production by the colonocytes. In contrast, at concentrations above 65 $\mu$M, NaHS severely inhibits colonocyte oxygen consumption by inhibiting the mitochondrial cytochrome $c$ oxidase activity [14*] (Fig. 3). Interestingly, inhibition of the mitochondrial cytochrome $c$ oxidase activity in human colon cancer

**FIGURE 2.** Mitochondrial oxidation of $\text{H}_2\text{S}$ in mammalian cells. This schematic representation recapitulates the mitochondrial metabolic pathways allowing the oxidation of endogenous and low concentrations of exogenous luminal $\text{H}_2\text{S}$ (nanomolar to low micromolar) in colonic epithelial cells. ETHE1, persulfide dioxygenase; $\text{H}_2\text{S}$, hydrogen sulfide; $\text{S}_2\text{O}_3^{2-}$, thiosulfate; $\text{SO}_3^{2-}$, sulfate; SQR, sulfide quinone reductase.

**FIGURE 3.** Effects of high $\text{H}_2\text{S}$ concentrations on mitochondrial energy metabolism in mammalian cells. High exogenous luminal concentrations of $\text{H}_2\text{S}$ (high micromolar to millimolar) originating from the metabolic activity of the intestinal microbiota inhibit the cytochrome $c$ oxidase (complex IV) activity in the mitochondrial respiratory chain, thus inhibiting oxygen consumption and mitochondrial ATP synthesis. $\text{H}_2\text{S}$, hydrogen sulfide.
cells by high (millimolar) concentration of NaHS results in a spectacular increase of the capacity of these cells to utilize glucose in the glycolytic pathway [17]. Thus, the high capacity of colonocytes for H₂S oxidation should be considered as a way for these cells to detoxify this bacterial metabolite and as a way to recover energy from it. The fact that colonocytes are among the most efficient cells for H₂S disposal is not surprising taking into account that these cells face the highest H₂S concentrations in the body [12]. Interestingly, the consumption of a high-protein diet in the rat model which increases the colonic content of H₂S coincides with an increased expression of the gene corresponding to SQR, the first and rate-limiting enzyme for H₂S detoxification [14], thus suggesting that the H₂S-detoxifying enzymatic system can adapt, up to a certain extent, to an increased amount of this bacterial metabolite within the colonic content. Recently, it has been shown that proanthocyanidin-containing polyphenol extracts originating from various plants can largely prevent the inhibitory effects of NaHS on human colonocyte oxygen consumption [18]. As proanthocyanidins are little absorbed in the small intestine, this result raises the possibility to reduce the concentration of H₂S in the large intestine by unabsorbed dietary compounds, thus avoiding the deleterious effects of this bacterial metabolite when present in excess in the luminal content.

Regarding the effects of H₂S produced endogenously within colonocytes, although little has been investigated regarding this aspect, it appears unlikely, notably in regards to what has been shown in other cell types [16], that the endogenous production of H₂S would reach a rate of production that would inhibit mitochondrial ATP synthesis in colonocytes.

Effects of exogenous and endogenous hydrogen sulfide on colonocyte proliferation and cell signaling

In accordance with what has been shown regarding the bell-shape response of colonic epithelial cells to NaHS in terms of mitochondrial energy metabolism, endogenously produced H₂S supports the maintenance of cellular energetics in normal colonocytes as well as colon cancer cell growth [19]. However, increase of H₂S concentration obtained with H₂S-releasing agents has been shown to inhibit mitochondrial oxygen consumption and proliferation of colonocytes originating from colon cancer [9,19].

The signaling effects of H₂S in mammalian cells are related to its role as a second messenger that transduces signals by interaction with cellular target proteins, including heme proteins, and through cysteine persulfidation that alters protein structure and function [17]. In the colonocytes, the information on the effects of exogenous and endogenous H₂S on signaling pathways is limited [9]. H₂S at high concentrations may inhibit proliferation of colonocytes by the activation of the AMP-activated protein kinase/mammalian target of rapamycin cascade. The positive effects of low concentrations of H₂S on human colon cancer cell proliferation and migration appear to be related to the stimulation of the serine/threonine protein kinase/phosphatidylinositol 3-kinase-signaling pathway and decrease of the p21 gene expression and interaction with NO [9,16].

Effects of exogenous and endogenous hydrogen sulfide and intestinal inflammation

The concept that H₂S, when present in excess in the colon luminal content, may increase the risk of mucosal inflammation has been proposed decades ago. Intracolonic instillation of NaHS (between 0.5 and 1.5 mM) in anesthetized rats for 1 h increased in colonocytes the expression of inflammation-related genes, namely, those corresponding to inducible nitric oxide synthase and interleukin-6 [14], showing that an excessive H₂S luminal concentration contributes to the process of colonic mucosal inflammation. In contrast, the endogenous synthesis of H₂S, which is believed to generate much lower intracellular concentration of H₂S, and low exogenous concentration of H₂S has been associated in different studies with an anti-inflammatory effect on the intestinal mucosa. Wallace et al. [20] reported that inhibition of endogenous H₂S synthesis in rats resulted in mucosal inflammation, suggesting that a limited amount of H₂S is necessary for limiting the risk of colonic mucosal inflammation [9].

The slow-releasing sulfide donor GYY4137 was found to attenuate in vitro, the lipopolysaccharide or TNF-α/IFN-γ induced increased permeability of colonocyte monolayer [21]. In addition, in an in vivo model of endotoxemia, the intraperitoneal injection of the sulfide-releasing agent GYY4137 displayed protective effect on the intestinal barrier dysfunction [21]. Similarly, the intraperitoneal injection of NaHS decreases the colonic epithelial injury associated with maternal separation in neonatal mice [22].

Recently, it has been hypothesized that H₂S could modulate intestinal inflammation through interactions with the protective mucus layer that limits direct interaction between bacteria and epithelial cells. Ijssennagger et al. [23] proposed that microbiota-generated H₂S could destabilize the protective mucus layer through the reduction of disulfide bounds linking the mucin 2 network. In
contrast, endogenous H$_2$S production by CSE contributes to the segregation between luminal bacteria and the mucosa through the production of mucus [20]. Clearly, new studies are needed to clarify the links between H$_2$S and the mucus layer and the consequences for intestinal inflammation.

The microbiota of new-onset pediatric Crohn’s disease patients is characterized by a high abundance of Atopobium, Fusobacterium, Veillonella, Prevotella, Streptococcus, and Leptotrichia, members of those genus being known to produce H$_2$S through the fermentation of sulfur-containing amino acids [24**]. Interestingly, in this cohort, the abundance of H$_2$S producers predicted inflammation severity and this observation was specific to cysteine degraders and not sulfate reduction. To investigate the causal-effect relationship between cysteine degrading bacteria and intestinal inflammation, the authors colonized IL10$^{-/-}$ mice with the H$_2$S producer Atopobium parvulum and observed a worsening of colitis, whereas this effect was attenuated by the administration of bismuth, an H$_2$S scavenger. Interestingly, in the same human cohort, the colonic mucosal biopsies from Crohn’s disease patient displayed decreased expression of the proteins involved in the mitochondrial H$_2$S detoxification (ETHE1, thiosulfate sulfurtransferase, SQK) [24**]. These latter results suggest that the impairment of H$_2$S detoxification system might amplify the toxic effects of the observed overproduction of H$_2$S by the microbiota from cysteine degradation.

Effects of exogenous and endogenous hydrogen sulfide and colorectal carcinogenesis

According to the analysis of gene expression and expression of related proteins in colon cancer and surrounding colonic mucosa samples, it appears that CBS, but not CSE or 3-MST, showed significant increase in their expression in the tumor when compared with the surrounding mucosa [19]. In both colonic tumors and surrounding tissues, the CSE displayed very low expression. As the colonic tumors and surrounding tissues are a complex mixture of different cell phenotypes, it would be necessary to document the expression of these enzymes in normal colonocytes recovered from both stem cells and from daughter cells with different level of differentiation. Data obtained regarding the expression of enzymes involved in H$_2$S production from cysteine in the HCT116 cells, which originate from a human colon cancer, indicate that these cells express CBS, 3-MST, and CSE enzymes [19].

Interestingly, two major H$_2$S-generating enzymes, namely, CBS and 3-MST, were found to be upregulated in the colonic HCT116 cancer cells which are resistant to the chemotherapeutic agent 5-fluorouracil, a situation that was concomitant with an increased capacity of the resistant cells to synthetize H$_2$S [25], thus suggesting that increased H$_2$S synthesis in cancerous colonocytes may increase their capacity to cope with chemotherapeutic drugs. Even more importantly, upregulation of CBS in human biopsies of precancerous adenomatous polyps was measured, and forced upregulation of CBS in adenoma-like colonic epithelial cells was sufficient for inducing metabolic characteristics and gene expression profile observed in CRC [26**]. In addition, the genetic ablation of CBS in mice resulted in the reduction of the number of mutagen-induced aberrant crypt foci. Experiments performed in nude mice bearing xenografts of either HCT116 cells (colonic cancer cells) or patient-derived tumor tissue indicate that inhibition of CBS reduces the growth rate of the tumor xenografts. This is likely related to the intratumoral effects of H$_2$S and/or paracrine mechanisms in the tumor microenvironment [27]. Taken as a whole, these results show that activation of the CBS/H$_2$S axis promotes colon carcinogenesis. Moreover, an increased H$_2$S production by the gut bacteria was predicted in colon cancer samples by using microbiota metabolism modeling system on the basis of bacterial relative abundance [11**]. The increased predicted H$_2$S production at tumor site was linked to Fusobacterium nucleatum abundance, a known H$_2$S producer suspected to promote CRC [11**]. Importantly, in this study, the dominant reaction predicted for increased H$_2$S production in CRC was the cysteine degradation pathway catalyzed by cysteine desulphydrase [11**].

Although the studies by Attene-Ramos et al. [28] suggested that H$_2$S is able to provoke DNA single and double breaks in mammalian cells, recent in-vitro and in-vivo works indicated that H$_2$S at concentrations found in the colorectal luminal content is unlikely to be genotoxic for colonocytes [14*].

CONCLUSION AND PERSPECTIVES

Among the numerous compounds produced by the metabolic activity of the intestinal microbiota toward amino acids, H$_2$S, mainly as a product of cysteine metabolism, has emerged as a conclusive example of the impact of the luminal content composition on the host colonic mucosa metabolism, physiology, and physiopathology. The capacity of the colonic epithelial cells to cope with changing H$_2$S luminal concentration by mitochondrial oxidation allows ATP production in the rapidly renewing colonic epithelium characterized by high-energy
demand. However, the capacity of colonocytes to oxidize \( \text{H}_2\text{S} \) is limited, and when the unbound luminal concentration of this gaseous mediator exceeds a threshold value, \( \text{H}_2\text{S} \) moves from an energy substrate to a metabolic troublemaker. In such a latter case, excessive \( \text{H}_2\text{S} \) can inhibit colonic epithelial cell proliferation by a decrease of mitochondrial ATP production because of the inhibition of the complex IV in the mitochondrial respiratory chain and by interfering with several signaling pathways in colonocytes. New experiments are clearly mandatory to evaluate to what extent, such an inhibition of colonic epithelial cells mitosis is detrimental for the process of colonic and rectal epithelium renewal, and how it may affect the epithelial homeostasis.

However, the colonocytes are not exposed only to the luminal \( \text{H}_2\text{S} \) at their apical side but can also produce this metabolite intracellularly. Although it is much likely that the endogenous production of \( \text{H}_2\text{S} \) and its metabolism in the oxidative catabolic pathway will lead to intracellular concentrations several order of magnitude below the ones that will be recovered from the luminal \( \text{H}_2\text{S} \), notably in case of high production of this bacterial metabolite, there is a paucity of information on the concentration reached intracellularly in different situations. This is mainly due to the fact that this parameter is not easily measurable notably because of its labile nature and the lack of simple specific methods of measurement.

Regarding the inflammatory process, there is some evidence that excessive luminal concentration of \( \text{H}_2\text{S} \) may participate in the inflammation, but a minimal amount of \( \text{H}_2\text{S} \), either from endogenous or luminal origin, appears to be protective against the inflammatory process in several studies. Last but not least, recent studies have indicated that in the process of colon carcinogenesis, cystathionine-derived \( \text{H}_2\text{S} \) is acting as a growth factor in cancerous colonocytes, and the activation of the CBS/\( \text{H}_2\text{S} \) axis promotes the growth rate of the colon tumor xenografts in experimental models.

**FIGURE 4.** Schematic recapitulation of the effects of \( \text{H}_2\text{S} \) in different situations, according to its origin and concentrations on colonocytes. (a) In healthy situation, the endogenous and exogenous \( \text{H}_2\text{S} \) colonocytes are oxidized by the SOU leading to mitochondrial ATP production. (b) When \( \text{H}_2\text{S} \) is produced by the microbiota in excess, \( \text{H}_2\text{S} \) inhibits the mitochondrial cytochrome c oxidase activity, and the synthesis of ATP by colonocytes. \( \text{H}_2\text{S} \) may in addition destabilize the protective mucus layer. This coincides with an increased expression of genes involved in the mucosal inflammatory processes. A minimum amount of the endogenous production of \( \text{H}_2\text{S} \) would limit the inflammatory process. Decreased expression of SOU is measured in colonic mucosal biopsies originating from pediatric Crohn’s disease patients. (c) In colorectal carcinogenesis, CBS activity is upregulated in precancerous adenomatous polyps, and stimulates the growth of cancerous colonic epithelial cells. In addition, increased production of \( \text{H}_2\text{S} \) by *Fusobacterium nucleatum* is suspected to promote colorectal cancer. CBS, cystathionine \( \beta \)-synthase; \( \text{H}_2\text{S} \), hydrogen sulfide; SOU, sulfide-oxidation unit.
Another important aspect that is worth to be taken into consideration and further developed is the possibility to modify the concentration of H$_2$S according to the dietary characteristics. For instance, the amount of H$_2$S in the colonic and cecal luminal contents can be increased in experimental rodent models when the amount of alimentary protein in the diet is increased, and there is new evidence that some unabsorbed dietary compounds can bind H$_2$S, thus reducing its free concentration. Thus, it should be feasible to control the intraluminal concentration of H$_2$S by dietary managements in situation of excessive production by the intestinal microbiota (Fig. 4) and thus to reduce its deleterious effects on the colonic mucosa.

New experiments with human volunteers in randomized double blind studies against placebo are mandatory to validate the efficiency of nutritional intervention for controlling H$_2$S luminal concentration in different physiological and pathological conditions.

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Conflicts of interest
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Papers of particular interest, published within the annual period of review, have been highlighted as:
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