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# Oxidation of hydrogen sulfide and methanethiol to thiosulfate by rat tissues: a specialized function of the colonic mucosa

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**Abstract**

Colonic bacteria release large quantities of the highly toxic thiols hydrogen sulfide ($H_2S$) and methanethiol ($CH_3SH$). These gases rapidly permeate the colonic mucosa, and tissue damage would be expected if the mucosa could not detoxify these compounds rapidly. We previously showed that rat cecal mucosa metabolizes these thiols via conversion to thiosulfate. The purpose of the present study in rats was to determine if this conversion of thiols to thiosulfate is (a) a generalized function of many tissues, or (b) a specialized function of the colonic mucosa. The tissues studied were mucosa from the cecum, right colon, mid-colon, ileum, and stomach; liver; muscle; erythrocytes; and plasma. The metabolic rate was determined by incubating homogenates of the various tissues with $H_2{}^{35}S$ and $CH_3{}^{35}SH$ and measuring the rate of incorporation of $^{35}S$ into thiosulfate and sulfate. The detoxification activity of $H_2S$ (expressed as nmol/mg per min) that resulted in thiosulfate production was at least eight times greater for cecal and right colonic mucosa than for the non-colonic tissues. Thiosulfate production from $CH_3SH$ was at least five times more rapid for cecal and right colonic mucosa than for the non-colonic tissues. We conclude that colonic mucosa possesses a specialized detoxification system that allows this tissue to rapidly metabolize $H_2S$ and $CH_3SH$ to thiosulfate. Presumably, this highly developed system protects the colon from what otherwise might be injurious concentrations of $H_2S$ and $CH_3SH$. Defects in this detoxification pathway possibly could play a role in the pathogenesis of various forms of colitis. 

*Keywords:* Hydrogen sulfide; Colonic; Mucosa; Oxidation; Thiolsulfate; Methanethiol

## 1. Introduction

Colonic bacteria produce large quantities of the highly toxic gases hydrogen sulfide ($H_2S$) and methanethiol ($CH_3SH$) [1]. Since the intestine is extremely permeable to $H_2S$ and $CH_3SH$ [1], severe tissue damage would be expected if the colonic mucosa did not possess an efficient means for detoxifying these gases.

High molecular weight thiols are detoxified via a methylation reaction catalyzed by thiol *S*-methyltransferase [2,3], and it has been commonly assumed that the colonic mucosa utilizes a similar mechanism to detoxify the low molecular weight thiols $H_2S$ and $CH_3SH$ [4,5]. However, we recently found that the cecal mucosa of the rat disposed of these compounds primarily via oxidation to thiosulfate [6]. The goal of the present study, carried out with rat tissues, was to determine if this ability to convert $H_2S$ and $CH_3SH$ to thiosulfate is (a) a specialized function of the cecal mucosa, or (b) a detoxification mechanism common to a variety of tissues.

## 2. Materials and methods

### 2.1. Tissues

Under pentobarbital anesthesia, tissue was obtained from five male Sprague–Dawley rats weighing 300–400 g. Tissues studied included mucosa obtained from the cecum, right colon, mid-colon, ileum, and stomach, as well as the liver, abdominal wall muscle, erythrocytes, and plasma. The gut segments were first rinsed with isotonic saline to remove all luminal debris, and then the mucosa was scraped off using the edge of a glass microscope slide. All tissue samples were maintained on ice until homogenized in ice-cold RPMI buffer solution (pH 7.4) in a ratio of 1 part tissue to 9 parts buffer (w/v). Homogenization was performed with a Duall® grinder with a Teflon pestle, using 8–10 strokes.

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### 2.2. Incubation studies

Fifty microliters of homogenate or buffer was added to a 20-mL polypropylene syringe fitted with a stopcock. (Preliminary studies showed that both $H_2S$ and $CH_3SH$ reacted rapidly with glass and most plastic surfaces, but reacted minimally with polypropylene.) After the syringes were heated to 37° in an incubator, 20 mL of $N_2$ containing approximately 150 ppm of $H_2{}^{35}S$ or $CH_3{}^{35}SH$ was added to the syringes, and the stopcocks were sealed. The $PO_2$ of the gas space averaged about 7 torr in these experiments. The syringes were incubated at 37° for exactly 10 min, at which time all gas in the syringes was immediately removed, and the syringes were placed in boiling water for 2 min to stop the reaction. An aliquot of the gas space from the $CH_3{}^{35}SH$ experiments was saved for analysis of $H_2S$. The 50 $\mu$L of homogenate (or buffer) was diluted with 0.5 mL of distilled water and subjected to sonification (Sonifier Cell Disruptor, model W185, Ultrasonics, Inc.) for 20 sec. A 50-$\mu$L aliquot was put aside for protein determination, and the remainder was used for the assay of radioactive sulfur products.

### 2.3. Synthesis of $H_2{}^{35}S$ and $CH_3{}^{35}SH$

Since neither of these gases is commercially available and their shelf-life is only a few days, it was necessary to synthesize these compounds on a daily basis as follows. Freshly passed rat feces were homogenized in a blender (Waring, Eberbach Corp.) using 1 part feces to 4 parts phosphate-buffered saline, pH 7.0. Fifty microcuries of [$^{35}$S]sodium sulfate or [$^{35}$S]cysteine (for $H_2{}^{35}S$ production) or [$^{35}$S]methionine (for $CH_3{}^{35}SH$ production) was added to 5 mL of fecal homogenate, and the mixture was incubated in a sealed 50-mL polypropylene syringe along with 40 mL of nitrogen. The gas space was removed at 24 hr, and $H_2{}^{35}S$ and $CH_3{}^{35}SH$ were isolated via their differential adsorption to MTO-Tenax TA (80/100 mesh, Supelco). The gas space was passed through a glass column containing Tenax (43 $\times$ 5 mm; 150 mg) maintained in dry ice. Both gases were adsorbed completely at this temperature. Then $H_2S$ and $CH_3SH$ were desorbed by heating the column to 25° and then 100°, respectively, as $N_2$ was passed through the column. The purity of the sulfur gas preparations was confirmed by gas chromatography.

### 2.4. Gas chromatography

Samples (0.30 mL) were analyzed for $H_2S$ and $CH_3SH$ using a gas chromatograph (model 5890, Hewlett Packard Co.) equipped with a Teflon column [8 ft. $\times$ 1/8 in., packed with Chromosil 330 (Supelco); maintained at 80° with a $N_2$ flow rate of 20 mL/min] and a sulfur chemiluminescence detector (model 355, Sievers Instruments, Inc.) that specifically detects sulfur-containing gases. The identity of the gases was verified initially using GC–mass spectroscopy, and subsequently via retention times. The gases were quantitated by comparison of peak areas with the areas of authentic standards.

### 2.5. Radioactivity and protein measurements

The radioactivity in the gas space was determined by adding 1 mL of gas to 0.3 mL of a 0.2 N solution of benzethonium hydroxide in methanol contained in a 5-mL polypropylene syringe. (Preliminary studies showed that both $H_2S$ and $CH_3SH$ were taken up avidly by this solution.) Then the benzethonium hydroxide solution was added to 10 mL of Ultima Gold, and radioactivity was determined via scintillation counting (Packard Instrument Co.). To determine the radioactivity of the homogenates, 0.1 mL of homogenate was added to 10 mL of Ultima Gold, and radioactivity was measured by scintillation counting. The protein content of the tissue homogenates was determined by the Coomassie protein assay (Pierce Chemical Co.), using bovine serum albumin as a standard.

### 2.6. HPLC

The metabolites produced during incubation of tissue with $H_2{}^{35}S$ and $CH_3{}^{35}SH$ were identified by HPLC (model C-R3A Chromatopac, Shimadzu) run at 2 mL/min and 2000 lb. pressure, using a 4 $\times$ 250 mm anion exchange column (IonPac AS16, Dionex Corp.) and a conductivity monitor (Amersham Pharmacia Biotech) for mass measurements. The eluant was 20 mM NaOH.

Protein was removed from the sonicated homogenate via filtration (Microncon centrifugal device, Millipore), and a 200-$\mu$L aliquot of the filtrate was mixed with 250 $\mu$L of solution containing 1 mM sulfate and 1 mM thiosulfate to serve as indicators of the elution times of these compounds. A 350-$\mu$L volume was injected onto the HPLC column, and 2.0-mL fractions were collected in individual scintillation vials. The radioactivity of these fractions was determined as described above. The identity of the $^{35}$S-labeled compounds was determined via comparison of their retention times to the retention time of the authentic sulfate and thiosulfate.

### 2.7. Calculation of metabolism

Metabolism of the labeled compounds by the homogenate was determined from the difference in the dpm eluting in the sulfate or thiosulfate peaks of the tissue-containing homogenate minus that observed in the buffer control. The rate of metabolism of $H_2S$ (in nmol/min) to sulfate and thiosulfate equaled:

$$\text{dpm converted to thiosulfate or sulfate/min per dpm/nmol of } H_2{}^{35}S\,. \quad (1)$$

The rate of metabolism of $CH_3SH$ (in nmol/min) to sulfate and thiosulfate equaled:

Table 1
Rate of production of sulfur-containing metabolites during incubation of homogenates of various rat tissues with $H_2S$ or $CH_3SH$

| Tissue | Rate of metabolite production from $H_2S$ (nmol/min per mg tissue protein) | | | | Rate of metabolite production from $CH_3SH$ (nmol/min per mg tissue protein) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | (N) | Thiosulfate | Sulfate | Total | (N) | Thiosulfate | Sulfate | $H_2S$ | Total |
| Cecal mucosa | 5 | 8.7 ± 2.4 | 0.92 ± 0.7 | 9.6 ± 2.4 | 5 | 3.4 ± 0.76 | 1.3 ± 0.05 | 1.5 ± 1.7 | 6.2 ± 1.2 |
| Right colonic mucosa | 2 | 7.6–10.9 | 0.64–0.65 | 8.2–11.6 | 5 | 2.1 ± 0.37 | 1.1 ± 0.06 | 2.3 ± 0.22 | 5.5 ± 1.2 |
| Mid-colonic mucosa | 5 | 4.2 ± 1.0 | 0.66 ± 0.08 | 4.8 ± 1.1 | 4 | 3.0 ± 0.87 | 1.2 ± 0.04 | 1.1 ± 0.22 | 5.3 ± 0.86 |
| Gastric mucosa | 5 | 0.54 ± 0.05 | 0.62 ± 0.03 | 1.2 ± 0.07 | 5 | 0.15 ± 0.036 | 0.29 ± 0.03 | 0.37 ± 0.09 | 0.81 ± 0.20 |
| Ileal mucosa | 5 | 0.44 ± 0.09 | 0.49 ± 0.05 | 0.94 ± 0.13 | 5 | 0.10 ± 0.02 | 0.33 ± 0.07 | 0.17 ± 0.09 | 0.60 ± 0.14 |
| Liver | 5 | 1.2 ± 0.20 | 1.2 ± 0.19 | 2.3 ± 0.39 | 5 | 0.62 ± 0.14 | 0.97 ± 0.09 | 0.37 ± 0.11 | 2.0 ± 0.39 |
| Muscle | 5 | 0.25 ± 0.06 | 0.17 ± 0.01 | 0.42 ± 0.06 | 4 | 0.046 ± 0.017 | 0.078 ± 0.01 | < 0.03 | 0.15 ± 0.06 |
| Plasma | 5 | 1.4 ± 0.21 | 0.33 ± 0.04 | 1.73 ± 0.26 | 4 | 0.088 ± 0.03 | 0.28 ± 0.08 | < 0.03 | 0.40 ± 0.14 |
| Erthyrocytes | 5 | 0.037 ± 0.01 | 0.12 ± 0.02 | 0.15 ± 0.02 | 4 | 0.013 ± 0.01 | 0.047 ± 0.01 | 0.08 ± 0.02 | 0.14 ± 0.02 |

Values are means ± SEM with the exception of the right colonic mucosa where N = 2 and data indicate the range of the observations.

$$\text{dpm converted to thiosulfate or sulfate/min per dpm/nmol of } CH_3{}^{35}SH\,. \quad (2)$$

The total rate of oxidation of $H_2S$ was assumed to equal the sum of the rates of production of sulfate and thiosulfate. Since $CH_3SH$ is converted to $H_2S$, and $H_2S$ is then metabolized to sulfate and thiosulfate, the rate of metabolism of $CH_3SH$ was calculated from the sum of the rates of production of sulfate and thiosulfate plus the net rate of $H_2S$ production.

## 3. Results

The rates of production of thiosulfate and sulfate during incubation of tissues with $H_2S$ are shown in Table 1. Cecal and right colonic mucosa oxidized $H_2S$ to thiosulfate at least eight times more rapidly than did any other non-colonic tissue, whereas the rate of production of sulfate was roughly equal for the various tissues. The total oxidation rate of $H_2S$ (thiosulfate plus sulfate production) was at least four times faster for right colonic mucosa than for any of the other tissues tested. Analysis of the gas phase upon completion of the incubations showed that no sulfur-containing gas other than $H_2S$ was present when $H_2S$ was metabolized.

The rates of production of thiosulfate, sulfate, and $H_2S$ during incubation of tissues with $CH_3SH$ are also shown in Table 1. As was the case with $H_2S$, this gas was metabolized to thiosulfate far more rapidly by cecal and colonic mucosa tissue than was the case with the other tissues tested. However, the production rate of thiosulfate by right colonic tissue was only about 30% as rapid as that observed with $H_2S$. A distinguishing feature of $CH_3SH$ metabolism by cecal and colonic mucosa was the appearance of appreciable quantities of $H_2S$, which represented about 20% of the total quantity of $CH_3SH$ metabolized. The total oxidation rate of $CH_3SH$ (thiosulfate plus sulfate and $H_2S$ production) was about 68% as rapid as was observed with $H_2S$ (see Table 1).

## 4. Discussion

The low molecular weight thiols, $H_2S$ and $CH_3SH$, have $LD_{50}$ values for rodents that are on the order of those for cyanide [7]. The acute toxicity of these compounds is thought to result from their ability to inhibit cytochrome oxidase via binding to the ferric moiety of cytochrome $aa_3$ [8]. In addition, $H_2S$ also appears to cause damage via mechanisms that are independent of cytochrome oxidase inhibition, such as hyperpolarization of potassium channels and alterations of neurotransmitters [9].

While the danger of exogenous (environmental) exposure to $H_2S$ and $CH_3SH$ has received considerable attention, it is not commonly recognized that there also is an appreciable endogenous exposure to these compounds via production by the colonic bacteria. The only available *in vivo* assessment of the production of these gases in humans consists of measurements carried out on gas passed per rectum [10]. These studies have shown concentrations of $H_2S$ and $CH_3SH$ ranging from 0.2 to 30 ppm. While such concentrations produce malodor, appreciable mucosal toxicity presumably would necessitate higher concentrations since irritation of membranes such as in the eye and the lungs requires concentrations of >20 ppm. Our studies in rats showed that $H_2S$ and $CH_3SH$ were absorbed very rapidly from the colon, suggesting that intracolonic concentrations of these gases might be substantially higher than gas passed per rectum [1]. Indeed, when gas was sampled from the rat cecum via chronically implanted cannulas, concentrations of up to 1000 ppm were observed for $H_2S$ [1]. Inhalation of 1000 ppm of $H_2S$ is lethal for rodents in less than 1 hr.

The production of $H_2S$ and $CH_3SH$ in the human colon has been assessed indirectly by measurements of the release of these gases during incubation of fecal samples. Feces from healthy subjects liberated $H_2S$ and $CH_3SH$ at rates averaging about 360 and 120 nmol/g dry weight per hr, respectively. Once again these values appear to minimize

the true exposure of the colon to these gases, since we found that the release of $H_2S$ and $CH_3SH$ per gram of rat cecal contents was >20 times the liberation rate of these gases by feces [1].

Analysis of sulfate balance in the intestine provides an indirect means of estimating the potential exposure of the human colon to $H_2S$. The intestinal mucosa is relatively impermeable to sulfate, and about 80% of dietary sulfate (2–15 mmol/day) reaches the colon [11]. Nevertheless, fecal sulfate (and sulfide) excretion is negligible relative to dietary intake, indicating that sulfate is being removed from the fecal stream during passage through the colon. Presumably, colonic bacteria reduce sulfate to sulfide, which is absorbed very rapidly. Based on this assumption, it can be calculated that the colon might be exposed to 1.6 to 12 mmol of $H_2S$/day, an exposure comparable to that resulting from the continuous inhalation of an atmosphere containing 36–280 ppm of $H_2S$. Since evacuation of a facility is recommended when the $H_2S$ concentration reaches 50 ppm, it is apparent that the quantities of $H_2S$ produced in the colon have an appreciable toxic potential. In reality, the exposure of the colonic mucosa to $H_2S$ could be greater than that calculated solely from sulfate since colonic bacteria very efficiently liberate $H_2S$ from a variety of substrates other than sulfate, such as mucin, taurocholate, and cysteine [12]. Small amounts of $CH_3SH$ are liberated during the bacterial metabolism of sulfate. In contrast, methionine is converted very efficiently to $CH_3SH$ by colonic bacteria.

The enormous exposure of the colonic mucosa to $H_2S$ and $CH_3SH$ suggests that this tissue must possess some mechanism to efficiently detoxify these compounds. Extensive literature exists on the detoxification of high molecular weight thiols via methylation [2,3], and it has been assumed that the colonic mucosa detoxifies the low molecular weight thiols $H_2S$ and $CH_3SH$ via a similar mechanism [4,5]. Such a reaction would metabolize $H_2S$ to $CH_3SH$, and then $CH_3SH$ would be converted to a relatively non-toxic gas, dimethylsulfide ($CH_3SCH_3$). However, we recently observed that when rat cecal mucosa was incubated with $H_2S$ or $CH_3SH$, there was no conversion of $H_2S$ to $CH_3SH$ or $CH_3SCH_3$ nor was $CH_3SH$ metabolized to $CH_3SCH_3$ [6]. Rather, at least in part, $CH_3SH$ was converted to $H_2S$, and $H_2S$ was converted to non-volatile metabolites. Further experiments demonstrated that this non-volatile metabolite was primarily thiosulfate. *In vivo* studies in which $H_2{}^{35}S$ was instilled into the lumen of the rat cecum showed that virtually all radioactivity leaving the cecum in venous blood was in the form of [$^{35}$S]thiosulfate. Thus, the cecal mucosa detoxifies $H_2S$ and $CH_3SH$, not via methylation, but rather by demethylation of $CH_3SH$ to $H_2S$ and oxidation of $H_2S$ to thiosulfate.

The purpose of the present study was to determine to what extent the detoxification mechanism that results in the rapid oxidation of $H_2S$ to thiosulfate is a specific adaptation of the cecal mucosa versus a general mechanism utilized by a variety of tissues that ordinarily would have very little exposure to $H_2S$ and $CH_3SH$. To this end, an assay was developed to measure the rate at which tissues converted $H_2S$ and $CH_3SH$ to the oxidative products, sulfate and thiosulfate. The tissues tested were liver, muscle, erythrocytes, plasma, and mucosa from the cecum, right colon, mid-colon, ileum, and stomach.

Cecal and right colonic mucosa metabolized $H_2S$ to thiosulfate about eight times more rapidly than did liver and about twenty times more rapidly than did ileal or gastric mucosa, muscle, or erythrocytes (see Table 1). The total $H_2S$ oxidizing activity (sulfate plus thiosulfate production rate) of the cecal and proximal colonic mucosa was about ten times greater than that of the gastric or small bowel mucosa and five times greater than that of the liver. This result differs from reported measurements of thiol *S*-methyltransferase activity, which have shown comparable activity in liver and colonic mucosa [3]. The rate at which cecal tissue oxidized $H_2S$ in our study (about 10 nmol/min per mg tissue protein) was about 100,000 times more rapid than the reported rate of $H_2S$ methylation by colonic tissue (100 fmol/min per mg protein) [4]. This finding is of some importance in that the reported methylation rate was well below the rate at which $H_2S$ is released in the rat cecum and hence would not detoxify an appreciable fraction of the $H_2S$ to which the cecum is exposed. In contrast, the oxidation rate observed in the present study was of the order required to metabolize all colonic $H_2S$ production. It seems unlikely that the low methylation rates of $H_2S$ observed by ourselves and others in colonic mucosa result from inhibitors of *S*-methyltransferase since we have observed appreciable methylation activity for high molecular weight thiols with similar mucosal homogenates. Rather, it appears that the *S*-methyltransferase enzymes of the colonic mucosa cannot effectively methylate $H_2S$.

As observed in our previous studies [6], the metabolism of $CH_3SH$ resulted in the appearance of $H_2S$ as well as thiosulfate and sulfate, indicating that the metabolic pathway involved, at least in part, includes the demethylation of $CH_3SH$ to $H_2S$ followed by oxidation of $H_2S$ to thiosulfate and sulfate. Demethylation activity, as measured by the sum of the accumulation rates of $H_2S$ plus thiosulfate plus sulfate, was about ten times higher in cecal and colonic mucosa than in gastric or ileal mucosa and three times higher than observed with liver (see Table 1). However, total demethylation activity of cecal and colonic mucosa was usually slightly less than the rate of oxidation of $H_2S$, suggesting that demethylation is the rate-limiting step in $CH_3SH$ detoxification. Presumably, *S*-methyltransferase does not perform this function since $CH_3SH$ is unlikely to be able to serve as a methyl donor for *S*-methyltransferase-catalyzed reactions. Mazel and coworkers [13] described a microsomal enzyme system that *S*-demethylates high molecular weight compounds, and it seems likely that this system could be responsible for the demethylation of $CH_3SH$ observed in the present study.

We conclude that the rapid oxidation of $H_2S$ and the

demethylation of $CH_3SH$ are not common characteristics of the entire gut mucosa, but rather specialized adaptations of the cecal and colonic mucosa. Presumably, these detoxification mechanisms were developed in response to the very high exposure of the colon to these two compounds.

The findings of Roediger and coworkers [14,15] have focused attention on the possibility that increased fecal production of $H_2S$ and $CH_3SH$ could play a pathogenic role in ulcerative colitis. These investigators showed that there is a defect in butyrate oxidation in the colonic mucosa of subjects with ulcerative colitis, and that this defect can be mimicked by exposure of the colon of experimental animals to high levels of sulfide. The colitis that develops in guinea pigs fed degraded carageenan (a non-absorbable, sulfate-rich polysaccharide) provides indirect support for the concept that excessive sulfide production may be injurious to the mucosa [16].

There have been multiple attempts to demonstrate that increased quantities of sulfide are produced in the colon of subjects with ulcerative colitis. In brief, comparison of feces from patients with ulcerative colitis and controls have shown that feces from patients with ulcerative colitis have: (a) a modestly increased fecal sulfide concentration in two studies [17,18] and normal concentrations in a third study [19]; (b) an increased rate of fecal release of $H_2S$ during incubation at 37° [12]; and (c) an increased activity of the bacterial enzyme system that reduces sulfate to sulfide [18].

It also is possible that sulfide production is normal in ulcerative colitis, but defective mucosal detoxification of $H_2S$ and/or $CH_3SH$ results in tissue injury. This defective detoxification could be hereditary, explaining the strong familial clustering of ulcerative colitis [20], or acquired due to some initiating form of mucosal damage, explaining the apparent tendency for ulcerative colitis to develop following a bout of infectious colitis. The detoxification activity of human mucosa biopsies obtained at colonoscopy can be readily assessed using the techniques developed for the present study. If decreased activity is observed, it will be necessary to determine if this defect is a cause or an effect of the colitis. A recent study [21] showed that oral administration of a non-absorbable bismuth preparation, bismuth subsalicylate, markedly reduced the fecal release of $H_2S$, and, to a lesser degree, $CH_3SH$, presumably via the binding of these compounds to bismuth. If defective metabolism is a cause (rather than an effect) of ulcerative colitis, reduction of the release of these compounds via the administration of bismuth subsalicylate would be expected to produce symptomatic benefit.

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## References

[1] Suarez FL, Springfield JR, Furne JK, Levitt MD. Production and elimination of sulfur-containing gases in the rat colon. Am J Physiol 1998;274:G727–33.
[2] Bremer J, Greenberg DM. Enzymatic methylation of foreign sulfhydryl compounds. Biochim Biophys Acta 1961;46:217–24.
[3] Keith RA, Otterness DM, Kerremans AL, Weinshilboum RM. *S*-Methylation of D- and L-penicillamine by human erythrocyte membrane thiol methyltransferase. Drug Metab Dispos 1985;13: 669–76.
[4] Weisiger RA, Pinkus LM, Jakoby WB. Thiol *S*-methyltransferase: suggested role in detoxification of intestinal hydrogen sulfide. Biochem Pharmacol 1980;29:2885–7.
[5] Pitcher MCL, Beatty ER, Harris RM, Waring RH, Cummings JH. Sulfur metabolism in ulcerative colitis: investigation of detoxification enzymes in peripheral blood. Dig Dis Sci 1998;43:2080–5.
[6] Levitt MD, Furne J, Springfield J, Suarez F, DeMaster E. Detoxification of hydrogen sulfide and methanethiol in the cecal mucosa. J Clin Invest 1999;104:1107–14.
[7] Sax NI. Dangerous properties of industrial materials. New York: Van Nostrand Reinhold Co., 1984.
[8] Nicholls P. The effect of sulphide on cytochrome $aa_3$. Isoteric and allosteric shifts of the reduced $\alpha$-peak. Biochim Biophys Acta 1975; 396:24–35.
[9] Ellenhorn MJ. Respiratory toxicology. In: Ellenhorn MJ, Schonwald S, Ordog G, Wasserberger J, editors. Ellenhorn's medical toxicology: diagnosis and treatment of human poisoning. 2nd ed. Baltimore: Williams & Wilkins, 1997. p. 1489–93.
[10] Suarez FL, Furne J, Springfield J, Levitt MD. Insights into human colonic physiology obtained from the study of flatus composition. Am J Physiol 1997;272:G1028–33.
[11] Florin T, Neale G, Gibson GR, Christl SU, Cummings JH. Metabolism of dietary sulphate: absorption and excretion in humans. Gut 1991;32:766–73.
[12] Levine J, Ellis CJ, Furne JK, Springfield JR, Levitt MD. Fecal hydrogen sulfide production in ulcerative colitis. Am J Gastroenterol 1998;93:83–7.
[13] Mazel P, Henderson JF, Axelrod J. S-Demethylation by microsomal enzymes. J Pharmacol Exp Ther 1964;143:1–6.
[14] Roediger WEW, Duncan A, Kapaniris O, Millard S. Reducing sulfur compounds of the colon impair colonocyte nutrition: implications for ulcerative colitis. Gastroenterology 1993;104:802–9.
[15] Roediger WEW. The colonic epithelium in ulcerative colitis: an energy-deficiency disease? Lancet 1980;ii:712–5.
[16] Onderdonk AB, Frankin ML, Cisneros RL. Production of experimental ulcerative colitis in gnotobiotic guinea pigs with simplified microflora. Infect Immun 1981;32:225–31.
[17] Florin THJ, Gibson GR, Neale G, Cummings JH. A role for sulfate-reducing bacteria in ulcerative colitis? Gastroenterology 1990;98: A170.
[18] Gibson GR, Cummings JH, Macfarlane GT. Growth and activities of sulphate-reducing bacteria in gut contents of healthy subjects and patients with ulcerative colitis. FEMS Microbiol Ecol 1991;86:103–12.
[19] Moore J, Babidge W, Millard S, Roediger W. Colonic luminal hydrogen sulfide is not elevated in ulcerative colitis. Dig Dis Sci 1998; 43:162–5.
[20] Satsangi J, Jewell DP, Rosenberg WCM, Bell JI. Genetics of inflammatory bowel disease. Gut 1994;35:696–700.
[21] Suarez FL, Furne JK, Springfield JR, Levitt MD. Bismuth subsalicylate markedly decreased hydrogen sulfide release in the human colon. Gastroenterology 1998;114:923–9.