Production and elimination of sulfur-containing gases in the rat colon

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Production and elimination of sulfur-containing gases in the rat colon. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G727–G733, 1998.—Highly toxic sulfur-containing gases have been pathogenetically implicated in ulcerative colitis. Utilizing a rat model, we studied the production and elimination of sulfur-containing gases within the unperturbed colon. The major sulfur-containing gases were hydrogen sulfide (H₂S), methanethiol, and dimethyl sulfide with cecal accumulation rates of 2.6, 0.096, and 0.046 µl/min, respectively. The dependence of H₂S production on dietary components was demonstrated via a sixfold reduction with fasting and a fivefold increase with carrageenan (a nonabsorbable, sulfur compound) feeding. Zinc acetate reduced cecal H₂S by fivefold, indicating the importance of H₂S binding by divalent cations. During passage from the cecum to the rectum, >90% of the sulfur gases were absorbed or metabolized. An H₂S turnover of 97%/min was observed in the isolated cecum. Thus mucosal exposure is >10 times the measured accumulation rate. Cecal mucosal tissue very rapidly metabolized H₂S and methanethiol via a nonmethylating reaction.

hydrogen sulfide; methanethiol; dimethyl sulfide; carrageenan; zinc acetate

THE COLONIC BACTERIA of humans and animals produce a variety of sulfur-containing gases, including hydrogen sulfide (H₂S), methanethiol (MES), and dimethyl sulfide (DMS) (9). These compounds are thought to be the main odoriferous agents in human flatus (10). In addition, these extremely toxic gases have been implicated in the pathogenesis of ulcerative colitis (1, 7).

Knowledge of the physiology of the sulfur-containing gases in the colon is presently limited to extrapolations from experiments employing cultures of isolated fecal organisms or fecal slurries. However, the rate of liberation of these gases from feces is a complex process that is presumably influenced by the numbers and activity of appropriate bacteria, availability of substrates, environmental factors such as PO₂ and pH, fecal consistency, and the intrafusal destruction of the gases via bacterial or nonbacterial reactions. It seems unlikely that conditions in fecal homogenates accurately simulate the in situ environment of the colon.

The goal of the present study carried out in rats was to obtain what appear to be the first reported measurements of the concentration and production of sulfur-containing gases within the unperturbed colon as well as the ability of the colon to eliminate these gases.

METHODS

Preliminary Sulfur Gas-Recovery Studies

Because the sulfur-containing gases are extremely reactive, initial studies were carried out to determine the most effective means of handling these compounds. These studies showed that H₂S and, to a lesser extent, MES reacted very rapidly with glass, rubber, and many plastic surfaces. In contrast, these gases were stable over several hours in polypropylene containers, which were used throughout the experiments.

Liberation of Gases in the Colon

Animals and surgical procedure. Seven male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing 350 ± 38 g were fitted with permanently implanted cecal cannulas, as described previously (5). A recovery period of at least 10 days preceded the beginning of experiments, which were carried out between the hours of 8 AM and 11 AM. In most studies, the diet was standard rat chow (Teklad rodent diet 8604; Harlan Teklad, Indianapolis, IN); however, in selected experiments the diet was supplemented with a poorly absorbed form of sulfate in the form of denatured carrageenan (3 g/l in drinking water) for a period of 21–49 days. In addition, some carrageenan-treated rats received zinc (which binds sulfhydryl compounds) in the form of zinc acetate, 1% wt/wt in standard rat chow.

Measurement of cecal concentration and volume of colonic gases. Using a 5-ml polypropylene syringe, we infused 3 ml of N₂ containing ~100 ppm of the very poorly absorbable gas sulfur hexafluoride (SF₆) through the cannula into the cecum. Mixing was obtained via repeated aspiration and reinfusion of the gas, and after 1 min the gas space was maximally aspirated (preliminary studies showed that equilibration of colonic sulfur gases with infused N₂ was complete by 1 min).

The quantity of each gas in the cecum was calculated from the formula

\[ \text{Volume} = [\text{gas}] \times 3 \text{ ml} \times \frac{\text{SF}_6\text{mol}}{\text{SF}_6\text{mol}} \]

Measurement of rate of accumulation of colonic gases. Rats were placed in a restraining cage and, via the cecal cannula, the colon was constantly perfused with N₂ (1.9 ml/min) using a syringe pump (Harvard Apparatus, South Natick, MA). A three-way stopcock attached to the cecal cannula permitted either infusion of gas or anaerobic collection of cecal gas samples. Gas arriving at the rectum was collected via a Foley catheter (Bard, Covington, GA) through which a polypropylene cannula was passed. The catheter was inserted 1.5 cm into the rectum, the balloon was inflated, and the polypropylene cannula was connected via a three-way stopcock to a polypropylene bag. All rectal gas collected over 10-min intervals was assayed as was a 3-ml aspirate from the cecum. The volume of gas in the bag, determined by aspiration into a calibrated syringe, always approximated the infusion rate (19 ml/10 min). In initial studies, the infused N₂ contained SF₆ as a dilution marker. When initial analyses of rectal gas in the steady state showed no appreciable dilution or concentration of SF₆, this gas was not employed in subsequent studies. The volume of a gas measured in these studies represents the net of production minus elimination and will be referred to as the “accumulation rate” of the gas. The rate that gases accumulated in the perfusate was determined from the product of the...
injection rate (1.9 ml/min) and the concentration of gases in the cecal and rectal aspirates.

Measurement of Disappearance Rate of Gases from the Colon

These studies were carried out under general anesthesia. A midline laparotomy was performed in five rats, fecal material was removed from the colon by stripping from the cecum to the rectum, and a cannula was inserted into the cecum via the terminal ileum. The colon was then perfused at a rate of 1.9 ml/min with a gas mixture made up of N₂ containing H₂S (40 ppm), MES (42 ppm), DMS (43 ppm), CO₂ (5%), and H₂ (5%). Gas was collected at the rectum via a polypropylene tube secured via a ligature. Preliminary studies in which pure N₂ was infused into the cecum showed that, with the exception of CO₂, the accumulation of the test gases in the cleansed colon was negligible relative to the infusion rates. The concentration of CO₂ was roughly 5% in the rectal collection. The disappearance rate of gases during passage through the colon was calculated from the difference between the infusion rate and the rate that gases passed the rectum [determined from the product of the infusion rate (1.9 ml/min) and the concentration of each of the various gases].

Measurement of Turnover of H₂S in the Cecum

Turnover of H₂S in the cecum was determined from the disappearance of H₂[35]S. Because Na₂[35]S is not commercially available, it was necessary to utilize fecal bacteria to synthesize H₂[35]S from Na₂[35]SO₄. We added 50–100 mCi of Na₂[35]SO₄ to a homogenate of rat feces (2 g of feces in 8 ml of NaCl, PO₄ buffer, pH 7.0). After 2 h of incubation at 37°C, the gas space, which contained H₂S, MES, and DMS, was aspirated through a column (0.3 cm × 10 cm) packed with Tenax maintained at −70°C via immersion in an ethanol-dry ice mixture. This column, which adsorbed all sulfur gases, was then warmed to 37°C and flushed with N₂. The initial 10 ml of effluent were shown by gas chromatography (GC) analysis to contain pure H₂S; this gas was used in subsequent experiments. The instability of H₂S required daily production of the labeled compound.

Under general anesthesia, the abdomen was opened, and the cecum was isolated between a ligature placed at the terminal ileum and a clamp across the cecal-right colon junction. We infused 2 ml of N₂ containing 100 ppm of SF₆ and ~1.1 µl of H₂S and 0.022 µCi of H₂[35]S into the cecum. Cecal contents were mixed via gentle palpation; then, at 1 min, a sample of cecal gas was aspirated for analysis for H₂S, H₂[35]S, and SF₆. To determine the importance of the cecal contents in H₂S turnover, the clamp was removed and all cecal contents were stripped out of the cecum. Only trace quantities of fecal material remained after this maneuver. The above-described procedure was then used to assess H₂[35]S turnover in the near absence of feces. To determine if H₂S turnover was saturable, in two studies the infusion was supplemented with superphysiological quantities of H₂S (725 µmol, yielding a concentration of 42,000 ppm), and disappearance of labeled and unlabeled H₂S was determined as described above. The fractional turnover of H₂[35]S per minute was determined from the formula

\[
\text{Fractional turnover} = \frac{([H_2^{35}S]_{\text{rec}}/ [H_2^{35}S]_{\text{inf}}) \times ([SF_6]_{\text{inf}}/ [SF_6]_{\text{rec}})}
\]

where the subscripts inf and rec refer to infused and recovered, respectively.

Measurement of Mucosal Metabolism of Sulfur Gases

In vitro studies were carried out with cecal mucosa and liver to determine the ability of these tissues to metabolize the sulfur-containing gases. Using a Duall tissue grinder, scrapings of cecal mucosa or segments of liver were homogenized in pH 7.4 RPMI 1640 buffer (insulin-transferrin-selenium media supplement; Biofluids, Rockville, MD) in a ratio of 5 mg tissue to 80 µl of buffer. We added 32 µl of this homogenate (2 mg of tissue) plus 10 ml of N₂ containing ~42 ppm each of H₂S, MES, and DMS to a 20-ml polypropylene syringe, which was incubated at 37°C. Buffer without tissue was incubated with the above-described gas mixture as a control. At 30 and 60 min, aliquots of the gas space were removed and analyzed for the three sulfur-containing gases. The reaction rate appeared to be roughly first order over the entire incubation period. Thus the rate of metabolism of a given gas by the tissues was determined from

\[
\text{Metabolic rate} = \frac{\text{ml of gas metabolized} \times \text{mg tissue}^{-1} \times \text{µM}^{-1}}{5}
\]

where µM refers to the logarithmic mean concentration of the gas during the assay period. Gas metabolized by tissue was assumed to equal the difference in disappearance rate of the gas from the gas space incubated with tissue vs. that of the buffer control.

Analytical Techniques

Colonic gas samples were assayed by diluting 1 ml of aspirate in 100 ml of argon. This diluted gas was used for assays for the sulfur-containing gases and H₂. A second 1 ml of aspirate was diluted with 4 ml of argon, and this gas was used for CO₂ and methane (CH₄) analysis.

The identities of the sulfur-containing gases were initially determined by GC-mass spectroscopy (70 eV; Kratos MS25) using a cross-linked silicone capillary column (30 m × 0.32 mm, HP-1; Hewlett-Packard, Palo Alto, CA). In subsequent studies the gases were identified (via characteristic GC retention times) and quantified using a GC (HP 5890A; Hewlett-Packard) equipped with a sulfur chemiluminescence detector specific for sulfur-containing compounds (model 355, Sievers Instruments, Boulder, CO). The gas sample (1.0 ml) was injected onto a column (2.4 m, 3.1 mm OD Teflon packed with Chromosil 330; Supelco, Bellefonte, PA) maintained at 80°C. The carrier gas was N₂ at a flow rate of 15 ml/min. H₂, methane, and CO₂ were analyzed as previously described (9). The concentrations of the gases were determined via comparison with curves constructed from known concentrations of authentic gases. Measurements of the radioactivity of H₂[35]S were obtained by adding 1 ml of cecal gas to 0.3 ml of 0.2 M of benzethonium hydroxide in methanol contained in a syringe. This mixture was then added to 15 ml of Ultima gold (Packard Instruments, Downer’s Grove, IL), which was counted in a liquid scintillation analyzer (1900 CA; Packard Instruments) for 10 min.

Data Analysis

Data, expressed as means ± SE, were analyzed by analysis of variance. To characterize individual group differences across treatments, post hoc Tukey’s honestly significant difference test was performed (Ref. 8 and version 5.2 SYSTAT for Macintosh; SYSTAT, Evanston, IL).
RESULTS

Volume of Sulfur Gases, H₂, and CO₂ in the Cecum

Table 1 summarizes the results of these studies. The only sulfur gases present in detectable concentration (≥0.02 ppm) in cecal gas samples were H₂S, MES, and DMS. Baseline measurements on the chow diet showed cecal volumes of H₂S, MES, and DMS of 2,690.3 ± 425.5, 96.1 ± 32.6, and 46.0 ± 27.8 nl, respectively. As shown in Fig. 1 and Table 1, a 36-h period of food deprivation significantly reduced the cecal volume of H₂S and DMS (but not MES). Supplementation of the chow diet with denatured carrageenan resulted in a roughly sixfold increase over baseline in H₂S (P < 0.001) but did not significantly alter MES and DMS volumes. The addition of zinc acetate to the diet of the carrageenan-fed animals resulted in a significant reduction in H₂S. Although H₂ and CO₂ volumes decreased with fasting, these differences did not reach statistical significance. Methane was not detectable (lower limit of detection was 0.3 ppm).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>H₂S, nl</th>
<th>MES, nl</th>
<th>DMS, nl</th>
<th>CO₂, ml</th>
<th>H₂, µl</th>
</tr>
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<tbody>
<tr>
<td>Baseline</td>
<td>8</td>
<td>2,690.3 ± 425.5*</td>
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<td>Fasting</td>
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<td>488.3 ± 104.1†</td>
<td>50.0 ± 10.9†</td>
<td>&gt;0.14 ± 0.1†</td>
<td>0.21 ± 0.04*</td>
<td>2.4 ± 0.7</td>
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<td>Carrageenan</td>
<td>4</td>
<td>14,205 ± 2,070‡</td>
<td>109.5 ± 8.6*</td>
<td>37.3 ± 4.7*</td>
<td>1.4 ± 0.1†</td>
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<td>Carrageenan + zinc acetate</td>
<td>4</td>
<td>3,736 ± 477.2*</td>
<td>74.4 ± 9.8†</td>
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<td>P</td>
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<td>0.028</td>
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<td>0.307</td>
</tr>
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Values are means ± SE. Data were analyzed by analysis of variance. H₂S, hydrogen sulfide; MES, methanethiol; DMS, dimethyl sulfide. Significant differences (P < 0.05) among treatment groups are denoted by different superscripts.

Accumulation Rate of Colonic Gases in the Cecum and Rate of Passage of These Gases per Rectum Assessed Via Constant Perfusion Technique

Figure 2 shows the rate of accumulation of each of the colonic gases in the cecum during the constant infusion experiments as well as the rate that these gases passed the rectum. The rate of accumulation of sulfur gases in the cecum fell appreciably during the 60-min study period, in contrast to the relatively constant rate of cecal accumulation of CO₂ and H₂. The rate of rectal passage of the sulfur gases during the early phases of this study was only a small fraction of the cecal appearance rate, indicating rapid removal of these gases during passage through the colon. In contrast, the accumulation rate of CO₂ and H₂ in the cecum was not significantly different from the rates that these gases passed the rectum.

Rate of Disappearance of Gases from the Colonic Lumen

Figure 3 shows the rate that gases infused into the cecum disappeared during passage from the cecum to the rectum. The rate of rectal passage of the sulfur-containing gases was only a small fraction of the cecal infusion rate. In contrast, after establishment of the steady state, the rates of rectal passage of CO₂, H₂, and CH₄ roughly equaled the infusion rate.

Rate of Turnover of H₂S in the Cecum as Assessed by H₂³⁵S Disappearance

Table 2 shows the fractional turnover rate of H₂³⁵S from the fecal-containing and cleansed cecum. More than 90% of the labeled compound disappeared from the gas space of the feces-containing cecum during the 1-min study period. A similar fractional disappearance rate was observed in the cleansed cecum. The elimination of H₂³⁵S also was >90%/min in two studies in which the labeled compound was infused with a superphysiological quantity of H₂S (725 µmol).

Study of Metabolism of Sulfur Gases by Colonic Mucosa

Figure 4 shows the GC tracings obtained at 0, 30, and 60 min of incubation of buffer or buffer containing 2 mg of

Table 1. Influence of dietary manipulation in volumes of cecal gases equilibrating with 3 ml of N₂

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>H₂S, nl</th>
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Values are means ± SE. Data were analyzed by analysis of variance. H₂S, hydrogen sulfide; MES, methanethiol; DMS, dimethyl sulfide. Significant differences (P < 0.05) among treatment groups are denoted by different superscripts.

Fig. 1. Influence of dietary manipulations on release of sulfur-containing gases in the rat cecum. A: hydrogen sulfide (H₂S). B: methanethiol (MES). C: dimethyl sulfide (DMS). Data are shown for the following diets: regular chow (filled bars), 36-h fasting (open bars), regular chow and 3% carrageenan in drinking water (hatched bars), and regular chow + 1% zinc acetate and 3% carrageenan in drinking water (crosshatched bars). Data are means ± SE. Different superscript letters signify significant (P < 0.05) differences.
of cecal mucosa or liver with a gas space containing ~42 ppm each of H2S, MES, and DMS. There was a slight fall in H2S and MES in the gas space incubated with buffer, a moderate decline when hepatic tissue was present, and virtually complete disappearance of these gases when cecal mucosal tissue was present. DMS concentration remained constant. During the first 30 min of the assay, the cecum metabolized H2S and MES at rates of 3,200 and 3,150 nmol·mg⁻¹·µM⁻¹, while the liver metabolized these two gases at rates of 425 and 500 nmol·mg⁻¹·µM⁻¹, respectively.

Table 2. Turnover of H2S in the cecum in 1 min

<table>
<thead>
<tr>
<th>Experiment</th>
<th>H2S at 1 min, µl</th>
<th>H2S³5S, µCi</th>
<th>Disappearance of H2S³5S, %/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cecum (with fecal contents)*</td>
<td>7.96 ± 5.95</td>
<td>0.18</td>
<td>89.5 ± 1.0</td>
</tr>
<tr>
<td>Cecum (empty)*</td>
<td>0.27 ± 0.05</td>
<td>0.018</td>
<td>97.3 ± 2.3</td>
</tr>
<tr>
<td>Cecum (empty)†</td>
<td>36.8 ± 7.45</td>
<td>0.06</td>
<td>94.0 ± 1.8</td>
</tr>
</tbody>
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Values are means ± SE. *H2S (2.0 µl) added at beginning of study. †Superphysiological quantities of H2S (214 µl) added at beginning of study.
appear to have been performed. This paucity of study reflects difficulties with the identification and quantitation of sulfur-containing gases as well as the unusual techniques required for gas production and elimination measurements in the gut. In the present study, the use of a chronically implanted cecal cannula and a detector specific and highly sensitive for sulfur-containing gases made it possible to study the in situ physiology of the sulfur-containing gases in the colon of the rat.

Analysis of gas obtained from the cecum or rectum revealed that only three sulfur-containing gases were present in detectable (>0.02 ppm) concentrations: H$_2$S, MES, and DMS, with H$_2$S being the predominant gas. These three gases also are the predominant sulfur-containing compounds in human flatus (10). The volumes of these sulfur gases responded variably to dietary manipulations (see Table 1). Fasting dramatically reduced H$_2$S and DMS, but did not significantly alter MES. Supplementation of the diet with carrageenan, a poorly absorbed organic sulfate obtained from seaweed, resulted in a massive increase in cecal H$_2$S volumes, but not in the other two gases.

The carrageenan data indicate that the availability of sulfur in the cecum is a major determinant of H$_2$S production. Potential sources of cecal sulfur include exogenous material such as dietary sulfate and sulfur-containing amino acids, both of which may be incompletely absorbed in the small bowel. Endogenous sources of sulfur include mucin and taurocholic acid. Each of these substrates supports production of either H$_2$S or MES in studies with fecal homogenates (4).

A variety of poorly absorbed divalent cations such as zinc, iron, and bismuth form insoluble salts with sulfide. The reduction of colonic H$_2$S when the carrageenan-containing diet was supplemented with zinc acetate exemplifies the potential regulatory role of these divalent cations in H$_2$S release. It seems possible that the well-known variability of flatus odor, which largely is determined by H$_2$S concentration (10), partially reflects the availability of sulfide-binding compounds in the fecal material. Dietary supplementation with a poorly absorbed divalent cation could provide a simple means of therapeutically reducing H$_2$S liberation in the colon.

The quantity of sulfur gases present at any instant in the gas phase of the colonic lumen is the net result of the rate that the gases are released from fecal material minus the rate that these gases are eliminated via absorption, metabolism, or excretion via the rectum. The net of these processes, which we have termed the accumulation rate, was determined by analysis of cecal and rectal gas obtained during a constant intracecal infusion of N$_2$. The accumulation rate of sulfur gases in the cecum was ~10 times more rapid than the rate that these gases passed the rectum. Because the gas volume of the cecum and colon was no more than 7 ml and the infusion rate was 1.9 ml/min, the mean transit time from cecum to rectum should be only 3–4 min. Thus the absorption/destruction rate of these gases is very rapid relative to their production in the extracecal colon. In contrast, the rates that H$_2$ and CO$_2$ accumulated in the cecum approximated the rates that each of these two gases passed the rectum. Because CO$_2$ concentration was ~5% in both cecal and rectal gas, the similar rates of CO$_2$ accumulation may simply reflect the tendency of this gas to rapidly equilibrate with the blood P$_{CO_2}$. However, the similar rates that H$_2$ passed the cecum and rectum suggest negligible production and absorption of this gas in the colon distal to the cecum.

To better assess the elimination rate of sulfur gases in the colon, studies were carried out in the colon cleansed of fecal material in which a cecal perfusion
provided the sole source of the sulfur-containing gases. About 90% of the intraceally administered sulfur-containing gases were eliminated during passage through the colon, while there was minimal reduction of CO₂, H₂, and CH₄. Thus the sulfur-containing gases are absorbed or destroyed at a rate that far exceeds the rate of absorption of the other colonic gases. Given the extraordinarily rapid clearance rate of sulfur gases, the odor of rectal gas probably will be strongly influenced by the colonic transit time as well as the absolute production rate of these gases.

To determine the absolute production rate of H₂S in the cecum, we measured the disappearance of H₂³⁵S from the isolated cecum. Over a 1-min period, 90% of the H₂³⁵S disappeared when the cecum contained feces and 97% disappeared when the cecum was cleansed of feces, indicating that the cecal mucosa rather than feces was responsible for the turnover of the labeled compound. The finding of a similar turnover rate when the tracer was infused with superphysiological quantities of H₂S indicated that the elimination of this gas was not readily saturable. The H₂S turnover of >90%/min indicates that the absolute production rate was at least 10 times greater than the quantity accumulating in the cecum over our 1-min study period (see Table 2). Thus exposure of the colon to these toxic compounds is much greater than would be predicted from static measurements of concentration or accumulation rate.

The rapid removal of the sulfur-containing gases from the colonic lumen could represent spontaneous oxidation or an enzymatically catalyzed mucosal reaction. In vitro studies were performed to determine if the cecum possessed an enzymatic mechanism to metabolize these gases. As shown in Fig. 4, cecal mucosa metabolized H₂S and MES at an extraordinarily rapid rate. This metabolism did not occur in heat-treated tissue. Liver tissue metabolized H₂S and MES about one-sixth as rapidly as did the cecal mucosa. Thus, although some spontaneous oxidation cannot be excluded, it is clear that the cecal mucosa possesses enzymes that permit the rapid detoxification of H₂S and MES.

In the majority of studies (3, 11), it has been assumed that the primary tissue detoxification mechanism for H₂S and MES is methylation catalyzed by thiol S-methyltransferase. This reaction converts H₂S to MES and MES to DMS. Studies (11) of rat tissues have shown that this enzyme system is widely distributed with the highest (and roughly equal) activities observed in colonic mucosa and liver.

The present study strongly suggests that the rapid colonic metabolism of H₂S and MES does not involve methylation. Because the final product of the S-methyltransferase-catalyzed reaction, DMS, was not metabolized by fecal tissue, an increase in DMS would have been observed if H₂S and MES were methylated to DMS. No such increase in DMS was observed (see Fig. 4). In addition, the activity of thiol S-methyltransferase is dependent on the availability of a methyl donor, S-adenosyl-L-methionine. Supplementation of the assay with this compound did not influence the rate that the colonic tissue metabolized H₂S or MES. Lastly, cecal mucosa metabolized H₂S about six times more rapidly than did hepatic tissue, whereas roughly equal activities of thiol S-methyltransferase activity have been reported for these two tissues (11).

Although excluding methylation, our studies do not clarify the nature of the enzyme system responsible for H₂S and MES metabolism. Metabolism of sulfide via a rhodanese-catalyzed reaction has been described (2); however, this reaction requires cyanide, which presumably was not available in our in vitro preparations. The apparent demonstration of an unrecognized detoxification reaction for H₂S and MES in colonic mucosa raises the possibility that defects in this enzyme system might play a role in the colonic toxicity of these compounds in conditions such as ulcerative colitis. This putative enzyme defect could be hereditary or acquired. Hereditary transmission would account for the high familial incidence of this condition. It also seems possible that mucosal damage occurring in the initial attack of ulcerative colitis, whatever the cause, could permanently reduce the H₂S-metabolizing ability of the mucosa, hence explaining the tendency for this disease to be a life-long affliction. This concept is readily testable via comparison of the H₂S- and MES-metabolizing ability of colonic mucosal biopsies. We previously observed (4) that the production of H₂S by feces of patients with ulcerative colitis was significantly greater than that of healthy controls, although there was appreciable overlap. It seems possible that tissue damage (i.e., ulcerative colitis) is restricted to individuals in whom high fecal H₂S production is associated with defective detoxification.

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