

Contribution of dietary protein to sulfide production in the large intestine: an in vitro and a controlled feeding study in humans¹⁻³

Elizabeth A Magee, Caroline J Richardson, Róisín Hughes, and John H Cummings

ABSTRACT

Background: Hydrogen sulfide is a lumenally acting, bacterially derived cell poison that has been implicated in ulcerative colitis. Sulfide generation in the colon is probably driven by dietary components such as sulfur-containing amino acids (SAAs) and inorganic sulfur (eg, sulfite).

Objective: We assessed the contribution of SAAs from meat to sulfide production by intestinal bacteria with use of both a model culture system in vitro and an in vivo human feeding study.

Design: Five healthy men were housed in a metabolic suite and fed a sequence of 5 diets for 10 d each. Meat intake ranged from 0 g/d with a vegetarian diet to 600 g/d with a high-meat diet. Fecal sulfide and urinary sulfate were measured in samples collected on days 9 and 10 of each diet period. Additionally, 5 or 10 g bovine serum albumin or casein/L was added to batch cultures inoculated with feces from 4 healthy volunteers. Concentrations of sulfide, ammonia, and Lowry-reactive substances were measured over 48 h.

Results: Mean (\pm SEM) fecal sulfide concentrations ranged from 0.22 ± 0.02 mmol/kg with the 0-g/d diet to 3.38 ± 0.31 mmol/kg with the 600-g/d diet and were significantly related to meat intake ($P < 0.001$). Sulfide formation in fecal batch cultures supplemented with both bovine serum albumin and casein correlated with protein digestion, as measured by the disappearance of Lowry-reactive substances and the appearance of ammonia.

Conclusion: Dietary protein from meat is an important substrate for sulfide generation by bacteria in the human large intestine. *Am J Clin Nutr* 2000;72:1488-94.

KEY WORDS Sulfide, meat, protein, sulfur amino acids, fermentation, urinary sulfate, fecal sulfide, intestinal bacteria, men

INTRODUCTION

Evidence that sulfide is toxic to colonocytes was reported in both in vitro and in vivo studies of sulfur metabolism (1). In the colonic environment, hydrogen sulfide is found in gaseous, dissolved, and anionic forms in a state of equilibrium determined by pH-related characteristics (2). Thus, $\text{H}_2\text{S} \leftrightarrow \text{H}^+ + \text{HS}^-$ ($\text{pK}_a = 7$) and $\text{HS}^- \leftrightarrow \text{H}^+ + \text{S}^{2-}$ ($\text{pK}_a = 12$). In the descending colon, the pH is ≈ 6 ; hence, sulfide is present mainly as hydrogen sulfide, either dissolved or as a gas. The exact conditions under which sulfides are toxic to epithelial cells are unknown, but several lines of experimental evidence implicate sulfide as a damaging agent

in ulcerative colitis. Perfusion of isolated rat colon for 4 h with 0.2–1.0 mmol sulfide/L increased mucosal apoptosis and goblet cell depletion (3). Roediger et al (4) showed inhibition of *n*-butyrate oxidation in vitro in both rat and human colonocytes at a concentration of 2 mmol/L (4). Using human colon tissue, Christl et al (3) showed that 1 mmol sulfide/L significantly increased cell proliferation rates and other changes normally seen in ulcerative colitis. Diminished *n*-butyrate oxidation was shown during perfusion of sulfide into the proximal rat colon (5, 6).

Although a variety of sulfur compounds are metabolized by gut bacteria, sulfide is generated in the human large intestine by 2 principal routes: first, by the action of sulfate-reducing bacteria on inorganic sulfur (sulfate and sulfite) (7) and second, through the fermentation of sulfur-containing amino acids (SAAs). The chief sources of sulfur in the diet are inorganic sulfate and the SAAs methionine, cysteine, cystine, and taurine. Sulfur also occurs naturally in the form of sulfur-containing glucosinolates in *Brassica* vegetables. Daily intake of inorganic sulfate is estimated to range from 1.5 to 16.0 mmol (8). Inorganic sulfur in the form of sulfite, sulfur dioxide, bisulfate, or metabisulfite is used routinely in the preservation of processed foods and beverages (9). SAA intake may vary with protein consumption. In addition to dietary sources, sulfated polysaccharides, such as mucin and chondroitin sulfate, are produced endogenously by the gastrointestinal tract.

No information is available on the total amount of sulfur consumed by humans today or the metabolic consequences of sulfur consumption, although amounts of dietary inorganic sulfate and SAAs are likely to be critical in determining sulfide production in the large intestine. The purpose of the current study was to examine the contribution of protein, mainly in the form of meat, to fecal sulfide in a human feeding study. Parallel in vitro experiments in batch culture systems were also performed to test the effect of different protein sources.

¹From the Dunn Clinical Nutrition Centre, Hills Road, Cambridge, United Kingdom, and the Department of Molecular and Cellular Pathology, University of Dundee, Ninewells Hospital and Medical School, Dundee, United Kingdom.

²Supported by the Medical Research Council and by a grant from the Ministry of Agriculture, Fisheries and Food.

³Address reprint requests to EA Magee, Department of Molecular and Cellular Pathology, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY, United Kingdom. E-mail: e.magee@dundee.ac.uk.

Received October 20, 1999.

Accepted for publication May 15, 2000.

TABLE 1
Foods and beverages provided in 3-d menu plans

Breakfast	Wheat biscuit cereal, milk, toast, margarine, and marmalade
Lunch	Bread, margarine, cheese, and tomato or chicken or beef; ketchup or pickle; digestive biscuits; and apple or orange
Evening	Egg or tuna or prawns with mayonnaise Vegetable or beef lasagna, sweet and sour vegetables or pork with rice, or beef steak with French fries and green beans Ice cream or cream with canned fruit
Beverages	Tea, coffee, or both with all meals and double-distilled water ad libitum
1-MJ increment ¹	50 g white bread, 15 g low-fat spread, and 20 g jam
Glucose beverage ²	0.5–1 bottle (170 mL/bottle)

¹Multiples of 1-MJ increments were added to or subtracted from the basic 10-MJ diet to meet the subjects' required energy intakes.

²Used to balance the 2-MJ variation in energy content between the diets containing 0 or 60 g meat/d and the diet containing 600 g meat/d.

SUBJECTS AND METHODS

Subjects

Five healthy men aged 36–49 y were recruited through the Dunn Clinical Nutrition Centre's volunteer register and advertisements in the local newspaper. All subjects had normal body weights, were healthy on the basis of a physical examination, and had no history of gastrointestinal disease. Subjects were not taking medication and had not been treated with antibiotics for ≥ 3 mo before the study starting date. The study protocol was fully explained to each subject before written consent was obtained. The study protocol was approved by the Ethical Committee of the Dunn Clinical Nutrition Centre, Cambridge.

Study design and diet composition

A partially randomized crossover design was used with five 10-d diets that varied in meat content. Meat intake ranged from 0 g/d with a vegetarian diet to 600 g/d with a high-meat diet, with intermediate amounts of 60 g/d, 240 g/d, and 420 g/d. A

3-d rotating menu was designed to provide a variety of foods throughout the study. The energy and, in most cases, macronutrient contents of the diets on the 3 menu days did not vary from the mean content by $>10\%$. The types of foods used throughout the study and the nutrient content of the diets are listed in **Tables 1** and **2**. The diets were isoenergetic and the 2-MJ variation in energy content between the 0- and 60-g/d diets and the 600-g/d diet was balanced with a low-electrolyte glucose drink (Hycal; SmithKline Beecham, Brentford, United Kingdom). Multiples of 1-MJ increments were added to or subtracted from the basic 10-MJ diet to meet the subjects' required energy intakes. Individual energy requirements for the volunteers were calculated with use of Schofield et al's (10) equations for estimating basal metabolic rate (BMR) for men aged 30–59 y, as follows: $BMR (MJ/d) = 0.048(\text{weight}) + 3.653$. The volunteers' levels of physical activity were also taken into consideration.

The protein, fat, and carbohydrate contents of the diets are shown in Table 2. Differences in the protein and fat contents of the 420- and 600-g/d diets were balanced almost entirely by carbohydrate (Hycal; SmithKline Beecham) in the 0- and 60-g/d diets. Protein intake in the 0- and 60-g/d diets was less than the average protein intake of 85 ± 20 g/d ($\bar{x} \pm SD$) for males in the United Kingdom (11), but met recommended dietary amounts (12, 13). Lean meat was used throughout the study. Fat intake contributed 28% of dietary energy (77 g/d) and did not vary by $>3\%$ between diet periods. Efforts were made to regulate micronutrient intake to dietary reference values advised by the Committee on Medical Aspects of Food Policy (12). Intakes of minerals that are rich in meat differed between the low- and high-meat diets and were not balanced, eg, iron intake ranged from 7 to 21 mg/d across the study diets.

Mean transit time and stool weight

During the 8 wk of this study, volunteers took 10 radioopaque plastic pellets with each meal (30/d) as balance markers and for gut transit determination, and collected all stool samples. Stools passed on days 1–8 of each test period were weighed, X-rayed for marker determination, and stored at -20°C for further analysis. On days 9 and 10 of each diet period, freshly passed stools were X-rayed and then used for total sulfide and sulfate determinations. Volunteers were given notebooks in which they kept a daily record of the times stools were passed and the times markers were

TABLE 2
Mean nutrient contents of the basic test diets containing 0, 60, 240, 420, or 600 g meat/d

Nutrient	0 g/d	60 g/d	240 g/d	420 g/d	600 g/d
Energy (MJ)	10	10	10	10	10
Protein					
(g/d)	51	64	121	165	212
(% of energy)	9	11	20	28	35
Fat (total)					
(g/d)	83	74	76	76	76
(% of energy)	31	28	28	28	28
Carbohydrate					
(g/d)	357	367	337	277	220
(% of energy)	61	63	56	46	37
Starch (g/d)	126	121	111	122	111
Total sugars (g/d)	127	119	109	105	106
Nonstarch polysaccharides (g/d)	13	13	13	14	14
Iron (mg/d)	7	8	15	18	21

taken. All frozen stool samples were weighed, and a mean stool weight corrected for marker output was calculated for fecal samples collected over the last 5 d of each test period (14). When marker recoveries were <100%, for example, when a volunteer forgot to take a dose of markers or forgot to collect a stool sample for the purpose of transit time calculations, marker outputs were corrected for missing markers. A single stool from the last 5 d of each diet was freeze-dried and measured for dry weight.

Urinary and fecal sulfates

Twenty-four-hour urine collections were obtained on days 9 and 10 of each diet period by using standard procedures (15). Boric acid was added to containers as a preservative. Volunteers were asked to take *p*-aminobenzoic acid (PABA) tablets on urine collection days and samples were analyzed to check for completeness (16). Volunteers were asked to report any problems with collection such as spillage or missed specimens during the 24-h period. Urinary volumes were recorded and aliquots were frozen immediately at -20°C for measurement of total sulfate. Urine samples with PABA recoveries <85% were excluded from any further analysis.

Isocratic separation of sulfate and sulfite (17) was carried out with a DX 500 ion chromatograph (Dionex, Camberly, United Kingdom) with a 50- μL injection loop, an IonPac AG9-SC guard precolumn (p/n 43186; Dionex), and an IonPac AS9-SC analytic column (p/n 43185; Dionex). The chromatograph was equipped with a self-regenerating suppressor and an electrical conductivity cell (both from Dionex). The eluent was a mixed solution of 1.8 mmol $\text{Na}_2\text{CO}_3/\text{L}$ (BDH, Lutterworth, United Kingdom) and 1.7 mmol NaHCO_3/L (BDH) in double-deionized water at a flow rate of 1.6 mL/min. Two-liter bottles of eluent were degassed with helium before analysis. Samples contained in 5-mL or 0.5-mL polyvials (Dionex) were loaded onto an automated sampler (Dionex) and 50 μL was injected onto the columns. Chromatograms were stored and viewed on a personal computer with the use of PEAKNET software (Dionex).

The measurement of total sulfate in urine requires an acid hydrolysis step to release *O*- and *N*-ester sulfate. A similar protocol was adopted for measurement of total sulfate in feces and diets. First, 0.75 mL of a thawed sample was hydrolyzed to liberate bound sulfate by heating the sample with 0.75 mL of 1.6 mmol HCl/L in a 90°C waterbath overnight. Hydrolyzed samples were then centrifuged for 5 min at $12000 \times g$ at room temperature (MSE Microcentaur centrifuge; Thistle Scientific Ltd, Glasgow, United Kingdom). Duplicate aliquots of filtered (2 μm , 13-mm diameter filters; Whatman, Abington, United Kingdom) supernates were removed and transferred to 5-mL polyvials (Dionex) and sealed with sealing film. Well-mixed polyvials were then capped and placed on an autosampler. In measuring sulfate in urine, feces, and diets, 10, 500, and 50 μL of the sample was added to 5.0, 4.5, and 5.0 mL eluent, respectively.

External standards were prepared volumetrically with solutions of sodium sulfate (Sigma, St Louis) and were treated in a manner identical to that outlined above for each sample type. All external standards and eluent solutions were prepared with double-deionized water to prevent contamination by sulfate in water. All standard calibration curves in this study were linear over the range of measurement ($r > 0.999$). The minimum level of detection in the diluted fecal sample was 5 $\mu\text{mol}/\text{L}$ and in urine was <1 mmol/L. Recovery experiments of samples (urine, feces, and food) supplemented with varying concentrations of sodium sul-

fate yielded recoveries not <98%. In the preparation and treatment of samples with small volumes, reproducibility was improved with the use of a positive displacement pipette (Biomaster; Eppendorf, Hamburg, Germany).

Measurement of fecal sulfide

We used the modified methylene blue method of Strocchi et al (18) as the basis for total acid-volatile sulfide determination in feces, subject to further validated modifications. To prevent losses of sulfide before analysis, all fecal slurries prepared in this study were fixed immediately in deoxygenated zinc acetate to avoid oxidation or other losses during storage. The measurement of a volatile compound such as sulfide is made difficult by the ease with which sulfide oxidizes on exposure to air and is absorbed by glass and plastic. In an effort to minimize sulfide loss, fecal samples were processed within 30 min of a stool being passed. Potassium phosphate buffer (0.1 mol/L, pH 7.0) was prepared by using double-deionized water in a 1-L Duran bottle (BDH) and was autoclaved (ST23; Dixons Surgical Instruments, Wickford, United Kingdom) at 120°C at 138 kPa (20 lbf/in²) for 20–30 min. The bottle was topped with residual deoxygenated buffer to exclude air and allowed to cool to room temperature for later use.

Fresh samples of feces were collected into a 177×304 mm polyethylene bag by means of a custom-made toilet-collecting device. A 10% (wt:vol) fecal slurry was prepared by mixing 10 g feces with 90 mL anoxic phosphate buffer. The slurry was then homogenized for 20 min (Colworth 3500 Stomacher; Seward Medical Ltd, London). The resultant homogenate was filtered through a 500- μm sieve (Endecotts Ltd, Chelmsford, United Kingdom) to remove solid food residue. Four milliliters of filtrate was then added to 1 mL of deoxygenated zinc acetate (120 g/L; Sigma) in a 7-mL glass bottle with a screw top and rubber seal. Hence, sulfide was trapped as zinc sulfide precipitate and the aluminium caps with rubber seals allowed the samples to be tightly sealed and stored for up to 6 mo at 4°C without loss of sulfide (19). Aliquots of well-mixed filtrate alone were stored in screw-top 1.5-mL microtubes (Sarstedt, Leicester, United Kingdom) at -20°C for measurement of total fecal sulfate.

Fecal batch cultures

To investigate HS^- formation by intestinal bacteria, a model batch culture system was set up with 2 protein substrates, bovine serum albumin (BSA) and casein. Fecal slurries (20%, wt:vol) were prepared by homogenizing freshly voided samples for 10 min in the following essentially sulfate-free, anaerobic (nitrogen purged) culture medium: 2.0 g $\text{KH}_2\text{PO}_4/\text{L}$, 4.5 g NaCl/L , 2.5 g KCl/L , 0.1 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}/\text{L}$, 0.001 g Haemin/L (Sigma-Aldrich, Poole, United Kingdom), and 0.0005 g vitamin B-12/L, pH 6.5. Next, 100 mL culture medium was mixed with either 5 or 10 g protein/L (BSA or casein) in the fermentation vessel (working volume: 280 mL). An equal volume of slurry was then added and the vessels were maintained under anaerobic conditions (oxygen-free nitrogen flow at 2.4 L/h) at pH 6.5 and 37°C for 48 h. At each of the 3 time points, 1-mL aliquots were taken for analysis (20).

Sulfide was trapped as a zinc sulfide precipitate in the samples by adding a deoxygenated solution containing 120 g zinc acetate/L (4:1). Hydrogen sulfide was collected from the precipitate by a microdistillation procedure (19) and trapped again in 1 mol NaOH/L . This enabled analysis by ion-exchange chromatography with amperometric detection (Ag/AgCl_2 working electrode). Culture supernates for protein and peptide estimations were precipi-

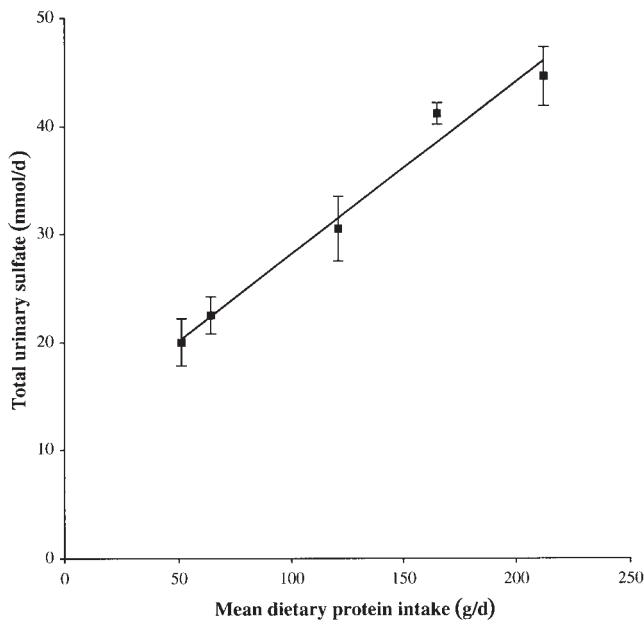


FIGURE 1. Mean (\pm SEM) total urinary sulfate output in 24-h collections. $n = 5$ for the diets containing 0, 60, 240, and 420 g meat/d and $n = 4$ for the diet containing 600 g meat/d. $y = 0.16x + 12.0$ ($r^2 = 0.78$, $P < 0.001$).

tated with 10% trichloroacetic acid and centrifuged at $20000 \times g$ for 10 min at room temperature. Protein (pellets resuspended in 1 mol NaOH/L) and peptides (supernates) were then measured by the Lowry method (21). Ammonia was measured spectrophotometrically with the phenol-hypochlorite method (22). Supernates were analyzed for sulfate by ion-exchange chromatography conductivity detection (Dionex DX-500) as described by Florin et al (17).

Statistical analyses

Statistical analyses were performed with SYSTAT for the Macintosh, version 5.2 (SPSS Inc, Chicago). Data are given as means \pm SEMs. Regression analyses were performed to investigate the relation between dietary protein intake (g/d), mean transit time, and stool weight and total urinary and fecal sulfate output and fecal sulfide concentrations. Fecal sulfide data were log transformed.

RESULTS

Subject compliance

Overall, subject compliance was good throughout the study. With the exception of one subject, all volunteers completed each of the 5 test diets. The subject who did not complete all 5 diets left the study in the early stages of the high-meat (600-g/d) diet after successfully completing 4 of the 5 diet periods. Data from this volunteer for the 0-, 60-, 240-, and 420-g/d diets are included.

All 24-h urine collections were deemed complete; PABA recoveries were $>85\%$ and the overall mean (\pm SEM) recovery was $102.2 \pm 4.0\%$. Mean (\pm SD) urinary volumes ranged from 1.9 ± 0.8 to 2.2 ± 0.9 L/d throughout the study and no significant effect of diet was observed.

The volunteers' body weights remained stable throughout the study, but minor adjustments to energy intake were required for

4 of 5 participants. Mean body weight deviated from start to finish by 1.8 kg with an overall mean (\pm SEM) of 65.7 ± 3.6 kg.

Dietary sulfate sources

The inorganic sulfate content of the study diets as measured by ion-exchange chromatography ranged from 2.72 mmol/d with the 0-g/d diet to 3.29 mmol/d with the 600-g/d diet. The overall mean sulfate intake throughout the entire study was 3.0 ± 0.23 mmol/d, with a CV not $>7.7\%$ between diet periods.

Fecal weight and whole-gut transit time

Individual daily stool output, corrected for fecal marker excretion, ranged from 62.5 to 238.6 g/d across the study diets. Mean (\pm SEM) stool weight for the group was 113 ± 23.4 g/d and no significant effect of diet was observed. Individual mean transit time ranged from 25 to 172 h across the diets with a group mean (\pm SEM) of 75.8 ± 18.6 h; there was no significant variance between dietary treatments.

Mean total sulfate in 24-h urine collections

Urinary sulfates (mmol/d) were measured for each volunteer in 2 urine collections at the end of each diet period. Increases in urinary sulfate with increases in protein intake were evident for all subjects (Figure 1).

Fecal sulfate

Mean (\pm SEM) concentrations of fecal sulfate for the group of volunteers ranged from 0.31 ± 0.06 mmol/kg with the 0-g/d diet to 0.52 ± 0.05 mmol/kg with the 600-g/d diet. Regression analysis showed no significant relation between dietary protein intake and fecal sulfate excretion ($r^2 = 0.01$, $P > 0.05$).

Fecal sulfide

Mean fecal sulfide concentrations correlated with mean protein intake (Figure 2).

Fecal batch cultures

The relation between sulfide production and the disappearance of protein in batch culture vessels fed 10 g BSA or casein/L is shown in Figures 3 and 4, respectively. The initial rates of sulfide production (\pm SEM) were 1.1 and $4.7 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g culture dry wt}^{-1}$ for 5 and 10 g BSA/L and 0.2 ± 0.1 and $0.9 \pm 0.8 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ for 5 and 10 g casein/L, respectively. In batch cultures fed casein, depletion of peptides present in the initial preparation was also evident, but when BSA was used as a substrate, there was no accumulation of peptides, suggesting that all residual protein broken down was utilized by bacteria.

DISCUSSION

The biology of sulfur in the human gut has escaped serious attention until recently. Thus, little is known about the amounts and sources of sulfur in the diet and about the subsequent digestion and absorption of sulfur from the intestine. However, the microbial metabolism of sulfur is well understood and in anaerobic ecosystems, such as the large intestine, reduced sulfur compounds such as hydrogen sulfide, which are highly toxic, can be formed. Sulfide has been implicated in the pathogenesis of ulcerative colitis (23, 24) and may damage the colonic epithelium in several ways. The purpose of this study was to begin to ascertain which dietary components contribute to sulfide generation in the

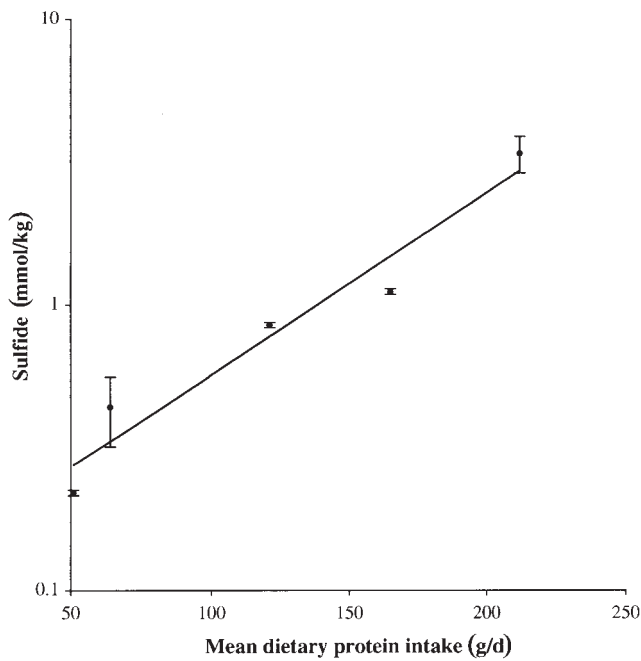


FIGURE 2. Mean (\pm SEM) fecal sulfide excretion in relation to dietary protein intake. $n = 5$ for the diets containing 0, 60, 240, and 420 g meat/d and $n = 4$ for the diet containing 600 g meat/d. $y = 0.2 \times 10^{0.002x}$ ($r^2 = 0.56$, $P < 0.001$).

large bowel. Because SAAs are probably the major source of sulfur in the diet, we examined their effect.

There are many difficulties associated with examining the effects of dietary components on health, and subject compliance is of major importance to the outcome of any dietary trial. It is extremely difficult to control for subject compliance completely, but live-in studies provide optimal opportunities for daily contact with volunteers and assessment of their compliance. In the present study, compliance with fecal and urinary collections was validated independently with use of radioopaque markers and PABA. Although one subject left in the early stages of the final test diet (the 600-g/d diet) because of concerns about consuming a high intake of beef, all other data from this volunteer were included and his compliance was good throughout the other diets. Overall, 97% of fecal markers taken were recovered in the volunteers' stool samples. All 24-h urine collections tested for PABA recovery had mean recoveries $>85\%$ and were consequently used for the measurement of total urinary sulfate.

The main finding of this study was the significant, dose-related increase in fecal sulfide concentrations with meat intake, confirmed by *in vitro* modeling of protein fermentation. The main dietary contributor to protein intake in the present study was meat, but this relation between sulfide concentrations and meat may hold true for any protein source. Meat provides a ready source of protein for use in experimental studies. Silvester and Cummings (25) showed that it is the amount of protein in the diet rather than its source that determines the amount of protein reaching the colon.

Reported concentrations of fecal sulfide vary according to the method of measurement and whether expressed as g dry or wet wt feces. Tangerman et al (26) reported mean sulfide concentrations of $0.8 \mu\text{mol/g}$ wet wt feces ($n = 7$) measured by head space gas chromatography. The methylene blue method has

been used most often, with mean wet weight fecal sulfide concentrations of $0.29 \mu\text{mol/g}$ ($n = 19$) (27), $0.66 \mu\text{mol/g}$ ($n = 14$) (28), and $1.6 \mu\text{mol/g}$ ($n = 8$) (17). Gibson et al (29) reported different fecal sulfide concentrations for nonmethanogens (based on breath-methane excretion of <1 ppm above room air) of 0.21 mmol/L ($n = 7$) and methanogens of 0.05 mmol/L ($n = 27$). The present study showed similar concentrations of sulfides in subjects eating average protein intakes, but showed that sulfide concentrations can increase dramatically with dietary change.

The mean fecal sulfide concentration of the present group of volunteers with the 600-g/d diet was 3.38 mmol/kg . Deleterious effects of sulfide within the human colon, such as mucosal ulceration, goblet cell loss, apoptosis, and distortion of the crypt architecture, have been observed with concentrations of $0.5\text{--}1 \text{ mmol/L}$ (3). Although the amount of meat consumed with the 600-g/d diet was much higher than the average UK intake of 150 g/d (EAM Magee, V Blokdijk, CJ Richardson, JH Cummings, unpublished observations, 1999), it is possible that a combination of dietary protein and sulfur oxoanion [S(IV)] additives in food may lead to fecal sulfide concentrations of this order.

The relevance of protein fermentation products in disease has received little attention to date, but the findings of this study make apparent that in this group of healthy volunteers the generation of fecal sulfide was related to dietary protein intake. However, fecal concentrations may not necessarily reflect production of sulfide in the human proximal colon. Furthermore, in the present study the extent to which fermentation products such as hydrogen sulfide were produced and reflected in fecal sulfide concentrations varied considerably between volunteers, but it is likely that these differences arose from variations in the type and activity of the gut microflora and bacterial activity. There was relatively little inorganic sulfate available for dissimilatory reduction by sulfate-reducing bacteria, but this may have resulted in low fecal sulfide concentrations for some individuals if low counts of amino acid-fermenting bacteria were present. It is also possible that for some individuals, both SAA fermentation and sulfate reduction by sulfate-reducing bacteria contributed to the generation of hydrogen sulfide. As expected (30), the change in dietary protein intake had no significant effect on bowel habits or transit time.

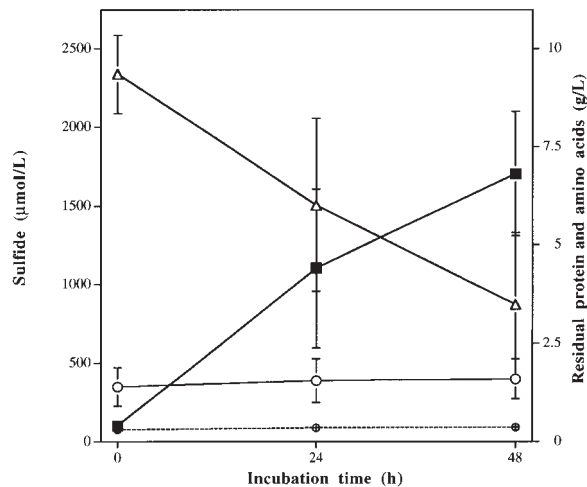


FIGURE 3. Mean (\pm SD) sulfide production in batch culture vessels to which 10 g bovine serum albumin/L was added ($n = 4$). ■, sulfide; ○, amino acids; Δ , residual protein; \oplus , sulfide in control vessel.

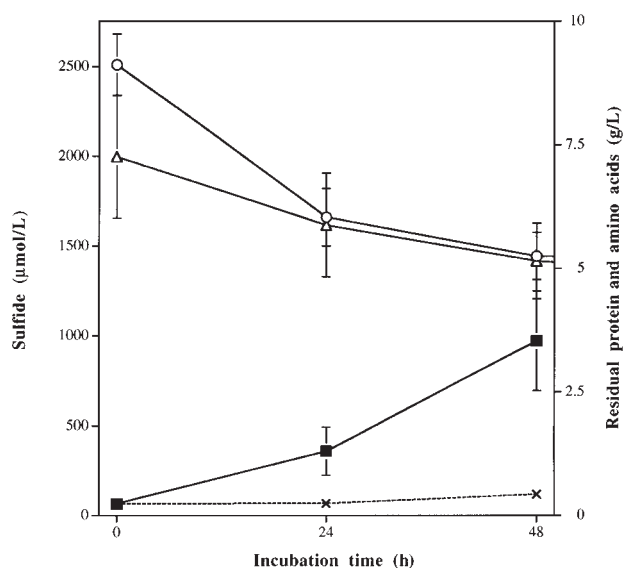



FIGURE 4. Mean (\pm SD) sulfide production in batch culture vessels to which 10 g casein/L was added ($n = 4$). ■, sulfide; ○, amino acids; △, residual protein; X, sulfide in control vessel.

As early as 1905, Wendt (31) emphasized the importance of measuring urinary sulfur in studies of protein metabolism. Several studies showed that most of the sulfur excreted as urinary sulfate in humans results from the oxidation of the SAAs methionine and cysteine absorbed in the intestine and generated from tissue breakdown (32). Animal proteins are rich in both methionine and cysteine. When methionine and cysteine are metabolized, sulfate is released and excreted in urine. The usefulness of urinary sulfate in determining human SAA requirements and as a reliable biomarker of protein intake (animal protein, $r = 0.69$; plant protein, $r = 0.54$; total protein, $r = 0.77$; and meat, $r = 0.50$) has been reported in the literature (33, 34). As with previously published data, this study showed a strong linear relation between total urinary sulfate excretion and total dietary protein intake ($P < 0.001$). Hence, total urinary sulfate excretion acted as a reliable marker of protein intake.

It has been shown that although most of the SAA sulfur is excreted as urinary sulfate, it is incorrect to ascribe urinary sulfate solely to oxidation of SAAs (31) in subjects fed British diets (15). Other dietary precursors of urinary sulfate could be inorganic sulfate and S(IV) compounds. With the 0-g/d diet (51 g protein/d), mean urinary sulfate was 20 mmol/d, which can be accounted for by an intake of 17 mmol SAAs (35) and 3 mmol dietary sulfate/d. Study diets were not measured for total S(IV) compounds. The percentage recovery of urinary sulfate from dietary SAA ranged from 100% with the 0-g/d diet to 68% with the 600-g/d diet. This lower efficiency may have been due to an increased spillover of protein into the gut, which could explain the disproportionate increase in fecal sulfide at the higher meat intakes. Other forms of fecal sulfur, such as bacterial protein, were not measured and may provide another route for sulfur excretion.

In a study of the role of the colon in sulfate absorption, sulfate intakes were measured against losses in feces and urine in a group of healthy subjects (17). Fecal sulfates for this group were uniformly low for all diets studied with a mean (\pm SEM) concentration of 0.42 ± 0.06 mmol/kg, ranging from 0.31 ± 0.06

0.52 ± 0.05 mmol/kg. As with stool weights and mean transit time, fecal sulfate outputs varied between subjects. The present findings suggest that fecal sulfate is not a major route for excretion of sulfur, even with high-protein diets. Further work is underway to establish the effect of sulfiting agents in the diet, in addition to dietary protein, on fecal sulfide concentrations in a controlled dietary study in healthy volunteers. 

REFERENCES

- Pitcher MCL, Cummings JH. Hydrogen sulphide: a bacterial toxin in ulcerative colitis? *Gut* 1996;39:1-4.
- Committee on Medical and Biologic Effects of Environmental Pollutants, Subcommittee on Hydrogen Sulfide. Hydrogen sulfide. Baltimore: University Park Press, 1979.
- Christl SU, Eisner HD, Scheppach W, Kasper H. Effect of sodium hydrogen sulfide on cell proliferation of colonic mucosa. *Gastroenterology* 1994;106:A664 (abstr).
- Roediger WEW, Nance S. Metabolic induction of experimental colitis by inhibition of fatty acid oxidation. *Br J Exp Pathol* 1986;67:773-82.
- Roediger WEW, Duncan A, Kapaniris O, Millard S. Sulphide impairment of substrate oxidation in rat colonocytes: a biochemical basis for ulcerative colitis? *Clin Sci* 1993;85:1-5.
- Moore JWE, Millard S, Babidge W, Rowland R, Roediger WEW. Hydrogen sulphide produces diminished fatty acid oxidation in the rat colon in vivo—implications for ulcerative colitis. *Aust N Z J Surg* 1997;67:245-9.
- 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.
- Florin THJ, Neale G, Goretzki S, Cummings JH. The sulfate content of foods and beverages. *J Food Comp Anal* 1993;6:140-51.
- Wedzicha BL. Chemistry of sulphur dioxide in foods. London: Elsevier Applied Science Publishers, 1984.
- Schofield WN, Schofield C, James WPT. Basal metabolic rate—review and prediction. *Hum Nutr Clin Nutr* 1985;39(suppl):1-96.
- Jackson AA, Margetts BM. Protein intakes in the adult population of the UK. *Int J Food Sci Nutr* 1993;44:95-104.
- Committee on Medical Aspects of Food Policy. Dietary reference values for food energy and nutrients for the United Kingdom. London: Her Majesty's Stationery Office, 1991. (Report on health and social subjects no. 41.)
- FAO/WHO/UNU. Energy and protein requirements. Report of an expert consultation. *World Health Organ Tech Rep Ser* 1985;724.
- Branch WJ, Cummings JH. Comparison of radio-opaque pellets and chromium sesquioxide as inert markers in studies requiring accurate faecal collections. *Gut* 1978;19:371-6.
- Bingham S, McNeil NI, Cummings JH. The diet of individuals: a study of a randomly-chosen cross section of British adults in a Cambridgeshire village. *Br J Nutr* 1981;45:23-5.
- Bingham S, Cummings JH. The use of 4-aminobenzoic acid as a marker to validate the completeness of 24 h urine collections in man. *Clin Sci* 1983;64:629-35.
- Florin THJ, Neale G, Gibson GR, Christl SU, Cummings JH. Metabolism of dietary sulphate: absorption and excretion in humans. *Gut* 1991;32:766-73.
- Strocchi A, Furne JK, Levitt MD. A modification of the methylene blue method to measure bacterial sulfide production in feces. *J Microbiol Methods* 1992;15:75-82.
- Richardson CJ, Magee EAM, Cummings JH. A new method for the determination of sulphide in gastrointestinal contents and whole blood by microdistillation and ion exchange chromatography. *Clin Chim Acta* 2000;293:115-25.

20. Macfarlane GT, Englyst HN. Starch utilisation by the human large intestinal microflora. *J Appl Microbiol* 1986;60:195–201.
21. Lowry OH, Rosebrough NJ, Lewis Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;143:265–75.
22. Solorzano L. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol Oceanograph* 1969;14:799–801.
23. Pitcher MCL, Beatty ER, Gibson GR, Cummings JH. Incidence and activities of sulphate-reducing bacteria in patients with ulcerative colitis. *Gut* 1995;36:A63 (abstr).
24. Roediger WEW, Duncan A, Kapaniris O, Millard S. Sulphide impairment of substrate oxidation in rat colonocytes: a biochemical basis for ulcerative colitis? *Clin Sci* 1993;85:1–5.
25. Silvester KR, Cummings JH. Does digestibility of meat protein help to explain large bowel cancer risk? *Nutr Cancer* 1995;24:279–88.
26. Tangerman A, Nagengast FM. A quantitative analysis of free hydrogen sulfide and bound sulfide in human feces. *Gastroenterology* 1994;106:A634 (abstr).
27. Pochart P, Dore J, Lemann F, Goderel I, Rambaud J-C. Interrelations between populations of methanogenic archaea and sulfate-reducing bacteria in the human colon. *FEMS Microbiol Lett* 1992;98:225–8.
28. Florin TH. Hydrogen sulphide and total acid-volatile sulphide in faeces, determined with a direct spectrophotometric method. *Clin Chim Acta* 1991;196:127–34.
29. Gibson GR, Cummings JH, Macfarlane GT, et al. Alternative pathways for hydrogen disposal during fermentation in the human colon. *Gut* 1990;31:679–83.
30. Cummings JH, Hill MJ, Jivraj T, Houston H, Branch WJ, Jenkins DJA. The effect of meat protein and dietary fiber on colonic function and metabolism. I. Changes in bowel habit, bile acid excretion, and calcium absorption. *Am J Clin Nutr* 1979;32:2086–93.
31. Wendt GV. Untersuchungen uber den eiweiss-und-salz-stoffwechsel beim menschen. (Investigations into protein and salt metabolism in man.) *Scand Arch Physiol* 1905;17:211–6 (in German).
32. Sabry ZI, Shadarevian SB, Cowan JW, Campbell JA. Relationship of dietary intake of sulphur amino-acids to urinary excretion of inorganic sulphate in man. *Nature* 1965;206:931–2 (letter).
33. Lakshamanan FL, Perera WDA, Scrimshaw NS, Young VR. Plasma and urinary amino acids and selected sulfur metabolites in young men fed a diet devoid of methionine and cysteine. *Am J Clin Nutr* 1976;29:1367–71.
34. Houterman S, van Faassen A, Ocke MA, et al. Is urinary sulfate a biomarker for the intake of animal protein and meat? *Cancer Lett* 1997;114:295–6.
35. Buss DH, Ruck NF. The amino acid pattern of the British diet. *J Hum Nutr* 1977;31:165–9.

