Methionine derivatives diminish sulphide damage to colonocytes – implications for ulcerative colitis

W E W Roediger, W Babidge, S Millard

Abstract

Background—Bacterial production of anionic sulphide is increased in the colon of ulcerative colitis and sulphides can cause metabolic damage to colonocytes. ***Aims***—To assess the reversal of the damaging effect of sulphide to isolated colonocytes by methionine and methionine derivatives.
Methods and subjects—Isolated colonocytes were prepared from rat colons and 12 human colectomy specimens. In cell suspensions $^{14}CO_2$/acetoacetate generation was measured from [1-^{14}C]-butyrate (5·0 mmol/l) in the presence of 0–2·0 mmol/l sodium hydrogen sulphide. The effect of 5·0 mmol/l L-methionine, S-adenosylmethionine 1,4 butane disulphonate and DL-methionine-S-methylsulphonium chloride on sulphide inhibited oxidation was observed.
Results—In rat colonocytes sodium hydrogen sulphide dose dependently reduced oxidative metabolite formation from n-butyrate, an action reversed in order of efficacy by S-adenosylmethionine 1,4 butane disulphonate>DLmethionine-S-methylsulphonium chloride>L-methionine. In human colonocytes S-adenosylmethionine 1,4 butane disulphonate most significantly improved $^{14}CO_2$ production (p=<0·005) suppressed by sodium hydrogen sulphide.
Conclusion—Sulphide toxicity in colonocytes is reversible by methyl donors. The efficiency of sulphide detoxification may be an important factor in the pathogenesis and treatment of ulcerative colitis for which S-adenosylmethionine 1,4 butane disulphonate may be of therapeutic value.

(*Gut* 1996; **39:** 77–81)

Cell Physiology
Laboratory,
Department of
Surgery and University
of Adelaide at The
Queen Elizabeth
Hospital, Adelaide,
Australia
W E W Roediger
W Babidge
S Millard

Correspondence to:
Associate Professor W E W
Roediger, Department of
Surgery, The Queen
Elizabeth Hospital,
Woodville, SA 5011,
Australia.

Anionic sulphur (SH- also termed sulphide) together with protein thiols and other sulphur species are essential for anaerobic bacteria of the fermentative organs to maintain anaerobiosis[1][2] and ensure production of short chain fatty acids.[3–5] Most sulphide in the colon is produced by the anaerobic sulphate reducing bacteria, which require sulphate[6] to facilitate removal of hydrogen produced by anaerobic bacteria in the colon,[7–9] a process in which methane producing bacteria act competitively.[10–12] The source of colonic sulphur is either ingested sulphate[13] or sulphur extracted transmucosally from the circulation by the intestine.[14–17]

The injurious potential of sulphur species in the colon was initially deduced from the ability of sulphated dextrans but not dextrans without sulphur to induce experimental colitis and colon cancer.[18][19] From a number of sulphur containing agents, sulphides proved most injurious to isolated human colonocytes[20] by a mechanism proposed in part to be due to persulphide formation of activated SCFAs.[21] Additional studies in humans showed that luminal sulphide concentrations in ulcerative colitis exceed that of control patients[22] and that ulcerative colitis patients produce more sulphide from fermentative substrates compared with control patients.[23] A role for sulphide in the causation of ulcerative colitis therefore seemed possible.

Protective mechanisms against the damaging effect of luminal sulphide exist in colonocytes. Methylation of sulphide[24][25] leads to the less toxic derivatives methanethiol and dimethylsulphide,[20] an action facilitated by thiol methyltransferases acting on S-adenosylmethionine (SAM) for which methionine is a precursor.[26] Thiol methyltransferase activity is high in human colonocytes[24][25] and methionine has been shown in preliminary rat experiments to be protective against the impairment of acute oxidative damage induced by sulphides in colonocytes.[21]

The aim of this study was to assess the remedial effect of methionine and methionine derivatives on suppression of oxidative metabolism produced by sulphides in rat and human colonocytes. The findings with sulphides and methionine are related to the disease process of ulcerative colitis.

Methods

Chemicals and reagents
Sodium n-butyrate was obtained from BDH (Melbourne, Australia) and sodium hydrogen sulphide (NaHS) from Ajax Chemicals (Sydney, Australia). L-Methionine was from Calbiochem (San Diego, USA). S-adenosylmethionine 1,4 butane disulphonate (SAMe), a stable salt of SAM was a gift from BioResearch Spa, Milan, Italy. DL-methionine-S-methyl sulphonium chloride (MMC) was obtained from Sigma, North Ryde, Sydney. [1-^{14}C]n-Butyrate was obtained from DuPont-NEN Research (North Sydney, Australia). Enzymes and co-factors for enzymatic estimation of metabolites were obtained from Boehringer Corporation (North Ryde, Australia) and Sigma Chemical Co (St Louis, MO, USA).

Tissue collection

Sprague Dawley rats were kept on a balanced diet and timed diurnal cycle in the Animal Houses of the University of Adelaide. Animals of the male sex between 200 and 250 g were used in the fed state and cells prepared from the entire colon constituting proximal and distal segments. Animals were killed by stunning/cervical fracture and the isolated colon flushed clear of luminal contents with 140 mmol/l NaCl. Isolated colonocytes were prepared as outlined previously.[27 28]

For human colonocytes segments of colon were obtained and prepared in an identical manner to that previously described.[20] Mucosa was obtained either from the ascending colon (proximal colon) or mid-descending colon to mid-rectum (distal colon) from 12 patients. Approval for experimentation was given by the ethics committees of The Queen Elizabeth Hospital and the University of Adelaide.

Substrates and incubation of isolated colonocytes

Experiments were conducted with isolated colonocytes freshly harvested and incubated within 30 minutes of collection.

Isolated cells were prepared and assessed as previously described[20] with the exception that dithiothreitol was omitted. Colonocytes maintained a linear rate of substrate oxidation for 60 minutes and no change in metabolic generation was seen with the addition of antibiotics. L-Methionine, SAM, MMC, and NaHS were freshly prepared in distilled water before incubation.

Cells suspensions, 1 ml representing 5-14 m dry weight of epithelial cells, were incubated for 40 minutes in conical flasks equipped with a glass centre well and stoppered with Suba-seals (William Freeman, UK). The gas phase was O_2 and CO_2 (19:1, vol/vol). Incubations were performed at 37°C in 1 ml of physiological saline containing 2-5% (wt/vol) bovine serum albumin and n-butyrate concentration of 5 mmol/l in all experiments. The specific activity of [1-^{14}C] n-butyrate was 1900 counts per minute (cpm)/µmol. Incubation of NaHS at concentrations of 0·5 to 2·0 mmol/l was performed simultaneously with radioactively labelled substrate. The incubation was stopped by adding 0·5 ml of 10% perchloric acid, and the protein precipitate was centrifuged after cooling with ice. The supernatant was neutralised to pH 7·4 with 20% potassium hydroxide.

Metabolic and radiochemical analysis of colonocytes

Acetoacetate concentrations were measured enzymatically according to Bergmeyer[29] from neutralised extracts of cells. Standards were included in each assay to ensure control between assays. The effect of NaHS on metabolite analysis was checked. No adverse or synergistic reactions were detected. $^{14}CO_2$ was trapped in 0·5 ml of 2 M NaOH injected into the centre well immediately after cell proteins were precipitated with perchloric acid. Flasks were gently shaken on ice for 1½ hours, and 0·1 ml of the solution was added to 5 ml of scintillant as previously described.[20] Samples were counted in a Beckman scintillation counter (Beckman Instruments, Irvine, CA), and counts were corrected for non-specific activity or volatility of the fatty acid n-butyrate.

Expression of results and statistics

Formation of metabolites by colonocytes was expressed per gram dry weight rather than unit protein, DNA or number of cells because production of hydrated mucus is variable and leads to clumping of cells. Dry weight of cells was obtained by drying 1 ml of suspension to constancy at 100°C and corrected for the dry weight of albumin contained in the medium. $^{14}CO_2$ generation from fatty acid was calculated from the specific activities and trapped $^{14}CO_2$ in sodium hydroxide.

Observations were obtained from the same tissue on which parallel experiments were performed and these were subjected to Student's paired t test, the 0·05 probability level was taken to mean a significant difference in observations.

Results

Sodium hydrogen sulphide acting on colonocytes of the rat dose dependently reduced CO_2 and acetoacetate production from n-butyrate (Table I) suggesting a global effect on fatty acid oxidation. L-methionine and the two methionine derivatives, SAMe and MMC, significantly improved β oxidation, that is both $^{14}CO_2$ and acetoacetate formation, affected by hydrogen sulphide particularly at concentrations of 1·0 mmol/l and higher (Table II). The improvement in β oxidation brought about was in the order of SAMe>MMC>L-methionine.

Human colonocytes collected from the prox-

TABLE I *Production of $^{14}CO_2$ and acetoacetate by isolated rat colonocytes from [1-^{14}C]-butyrate in the presence of NaHS and methionine derivatives. Mean (SEM) of five experiments each comprising pooled cells from four rats*

Conditions n-Butyrate (5 mmol/l) Additive		Metabolite formation (µmol/min/g dry wt) Presence of NaHS (mmol/l)				
		0	0·5	1·0	1·5	2·0
Nil	$^{14}CO_2$	2·25 (0·26)‡	1·76 (0·24)‡	1·09 (0·38)‖	0·68 (0·20)•	0·20 (0·06)
	acetoacetate	2·85 (0·23)*†	2·58 (0·18)*	2·08 (0·21)†¶	1·30 (0·25)	0·38 (0·09)
L-methionine	$^{14}CO_2$	2·22 (0·29)	1·87 (0·26)	1·41 (0·24)‖	1·34 (0·24)	0·89 (0·24)
	acetoacetate	2·62 (0·44)	2·90 (0·39)	2·55 (0·27)¶	2·58 (0·38)	1·65 (0·38)
SAMe	$^{14}CO_2$	2·08 (0·32)	1·69 (0·23)	1·37 (0·27)‖	1·36 (0·22)	1·28 (0·27)
	acetoacetate	2·57 (0·38)	2·30 (0·42)	2·07 (0·23)§	2·14 (0·34)•	2·39 (0·32)
MMC	$^{14}CO_2$	2·15 (0·31)	1·65 (0·20)	1·61 (0·23)‖	1·17 (0·21)	1·20 (0·21)
	acetoacetate	2·07 (0·33)	2·42 (0·32)	2·59 (0·39)¶	2·14 (0·41)	2·09 (0·35)

Student's paired t test: *NS, †$p<0·01$ and ‡$p<0·001$ compared with no NaHS. §NS, ‖$p<0·01$, and ¶$p<0·025$ compared with 1·00 mmol/l NaHS alone. •$p<0·01$ compared with 1·5 mmol/l NaHS alone.

TABLE II *Production of acetoacetate by isolated human colonocytes of distal colon from [1-¹⁴C] butyrate in presence of NaHS and methionine derivatives. Mean (SEM) of six specimens*

Conditions n-Butyrate (5 mmol/l)	Acetoacetate formation (μmol/min/g dry wt)		
	NaHS (mmol/l)		
Additive	0	1·0	2·0
Nil	2·37 (0·45)*	1·89 (0·55)†	0·53 (0·1)‡§
L-methionine (5 mmol/l)	2·18 (0·35)	2·05 (0·32)	1·65 (0·61)‡
SAMe (5 mmol/l)	1·72 (0·32)*	1·68 (0·38)†	1·61 (0·28)§

Student's paired *t* test: *†NS, ‡p<0·05, §p<0·025 compared with NaHS alone.

imal and distal colon exposed to sodium hydrogen sulphide showed a linear reduction in CO_2 production from n-butyrate and at all observed concentrations L-methionine significantly improved CO_2 production in colonocytes exposed to SH- (Fig 1). In these experiments comparison between methionine, SAMe, and MMC were not carried out nor was acetoacetate formation evaluated. These findings led to further experiments with human colonocytes with regionality of the colon a main consideration. Colonocytes from the distal colon showed that 2 mmol/l NaHS reduced butyrate derived $^{14}CO_2$ production (mean (SEM)) from 1·31 (0·23) to 0·38 (0·18) μmol/min/g (dry weight), which was increased to 0·99 (0·2) by 5 mmol/l of SAMe (Fig 2). Acetoacetate production was also improved by SAMe and methionine (Table II). SAMe was significantly better (p<0·01) than L-methionine in improving CO_2 production from butyrate by impaired human colonocytes (2·0 mmol/l NaHS).

Discussion

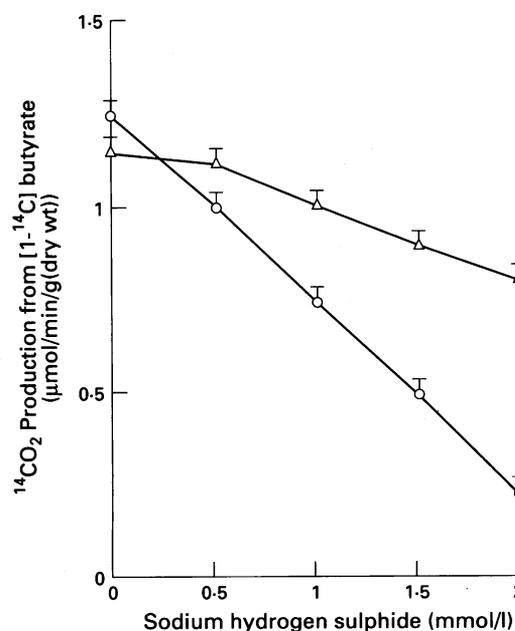The relation between the concentration of hydrogen sulphide and suppression of CO_2 production from n-butyrate by colonocytes of humans is now shown to be linear, compared with a more hyperbolic curve observed at the same concentration values of sulphide with cells of the rat.[21][30] This suggests that human colonocytes are less sensitive to the damaging effect of hydrogen sulphide than colonocytes of the rat. The median effective dose (ED) producing 50% reduction of butyrate respiration in human colonocytes was 1·2 mmol/l compared with about 6·0–9·0 mmol/l for the rat.[21][30] The ED50 level in humans might be higher in vivo where absorption or inactivation by detoxification might change the toxic effect of sulphides. Despite the oxidative environment of current experiments sulphide was still toxic indicating that oxidases against thiols are not very active in colonocytes of rat and humans. Current observations of acute exposure to sulphide may not reflect reactions that prevail under chronic exposure in vivo where cell responses could be modified by tachyphylaxis or change in enzyme activity of thiol methyltransferases in colonocytes.

In the colonic mucosa the free amino acid level of methionine is low compared with other amino acids[31] nevertheless the colonic mucosa extracts methionine from the circulation,[14][15] at a rate that is increased under conditions of surgical stress, for methionine, alanine, and glutamine but not other amino acids.[32] Very little methionine is normally absorbed from the colonic lumen of the mammalian colon.[33] In enterocytes and colonocytes methionine is the precursor for glutathione[34][35] that acts as an antioxidant[36] and also is the precursor of S-adenosylmethionine[26] that acts as an antireductant through its action of methylation[24][25] (Fig 3). The formation of S-adenosylmethionine in colonocytes requires adenosine triphosphate and methionine with appropriate enzymes.[37] Methionine that is not converted to SAM in bodily tissues is con-
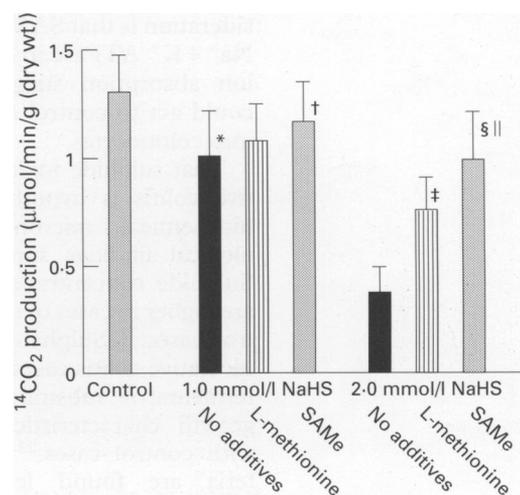

Figure 1: The rate of $^{14}CO_2$ production from [1-¹⁴C]-butyrate from isolated colonocytes of six human colons (three proximal colon, three distal colon) exposed to NaHS (o) or NaHS with L-methionine, 5 mmol/l (Δ) Student's paired t test at 0·5 mmol/l NaHS=p<0·001 and all higher concentrations of NaHS.

*Figure 2: Production of $^{14}CO_2$ by isolated human colonocytes of distal colon from [1-¹⁴C]-butyrate in presence of NaHS and methionine derivatives. Mean (SEM) of six cases. Student's paired t test: *p=<0·01 compared with control; †p=NS compared with no additives at 1·0 mmol/l NaHS; and at 2·0 mmol/l NaHS ‡p=<0·05 compared with no additives, §p=<0·01 compared with L-methionine, and ‖p=<0·005 compared with no additives.*
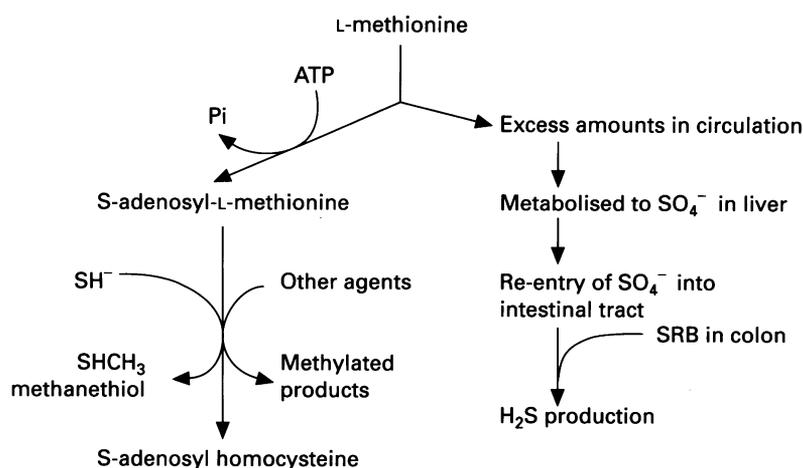
Figure 3: Aspects of L-methionine metabolism related to current studies with colonocytes. Both useful and harmful functions of L-methionine are highlighted as detailed in discussion.

verted to sulphate by hepatocytes[38][39] and the sulphate returned to the intestinal lumen by a rapid transport system that extracts sulphate from the circulation.[14-16] The sulphate in the intestinal lumen derived from the circulation will be subjected to fermentative action by sulphate reducing bacteria in the colon and increased sulphide production may result (Fig 3).

SAM is the universal methylating agent of sulphur, carbon, and nitrogen groups of various chemical compounds[37] and has been therapeutically used for the treatment of depression,[40] osteoarthritis,[41] and liver diseases.[40] The drug action is postulated to be through methylation processes of cell membranes or replenishment of depleted glutathione stores.[41][42] MMC has been successfully used to treat ulcerative colitis[43] and proposed to counteract the depleted glutathione levels found in the mucosa of ulcerative colitis.[44] Based on present observations methylation of toxic sulphides could also account for the utility of this drug in ulcerative colitis. Yet a further consideration is that SAM via methylation changes $Na^+ + K^+$ ATPase in colonocytes[45] to promote ion absorption suggesting that methylation could act to control entry of anionic sulphide into colonocytes.

That sulphide toxicity might initiate ulcerative colitis is hypothetical yet a number of biochemical, microbiological, and epidemiological findings support such a possibility. Sulphide concentrations in the colonic lumen are higher in cases of ulcerative colitis than control cases.[22] Sulphate reducing bacteria from ulcerative colitis cases produce more H_2S from fermentative substrates and have more robust growth characteristics than those compared with control cases.[23] Sulphate reducing bacteria are found less frequently in rural black population groups where the prevalence of ulcerative colitis is very low compared with the population groups in the United Kingdom[46][47] where the prevalence of ulcerative colitis is high. Biochemically sulphides selectively inhibit n-butyrate oxidation[21] the main nutrient of colonocytes and sulphides are more toxic to colonocytes of the distal

colon[20] where colitis is found more frequently. Present results show that sulphide has a graded damaging effect on colonocytes according to concentration of sulphide and that the damaging effect is partially reversible by methionine and SAMe. The most efficacious drug for ulcerative colitis, 5-ASA has now been shown to inhibit sulphide formation by colonic bacteria in humans.[48-50] The precise mode whereby sulphide might act in the pathogenesis of ulcerative colitis remains unknown – overproduction of sulphide or diminished detoxification each or both require investigation.

In considering either L-methionine or SAM for clinical use in ulcerative colitis, L-methionine would be undesirable for such purposes as adenosine triphosphate levels in colonocytes to generate SAM from methionine (Fig 3) are diminished in acute colitis[51] and consequently excess L-methionine would be converted to sulphate in the liver.[38] Re-entry of such sulphate into the intestinal tract[16] would increase sulphide formation in the colon. The functions of methionine with regard to colonocytes seems delicately poised between a useful and a potentially harmful role (Fig 3). The application of SAMe as a therapeutic agent is warranted in view of the current finding of protection against sulphide toxicity. The place of sulphide in the pathogenesis of ulcerative colitis requires further detailed investigation both at a cellular-biochemical and clinical-therapeutic level.

1 Atlas RM, Bartha R. The sulfur cycle. In: *Microbial ecology: fundamentals and applications.* 2nd ed. Menlo Park, CA: Benjamin Cummins Publishing, 1987: 342–58.
2 Fuchs G, Ecker A, Strauss G. Bioenergetics and autotrophic carbon metabolism of chemolithotrophic archaebacteria. *Biochem Soc Symp* 1992; **58:** 23–9.
3 Whanger PD, Matrone G. Effects of sulfur deficiency on metabolism in sheep. In: Muth DH, ed. *Symposium: Sulfur in nutrition.* Westport, CT: AVI Publishing, 1970: 153–64.
4 Goodrich RD, Garrett JE. *Sulfur in livestock nutrition.* In: Tabatabai MA, ed. Sulfur in agriculture. Madison, WI: American Society of Agronomy, 1986: 617–33.
5 Hegarty RS, Nola JV, Leng RA. Sulphur availability and the microbial fermentation in the fauna-free rumen. *Arch Anim Nutr* 1991; **41:** 725–36.
6 Widdel F. Microbiology and ecology of sulfate-and sulfur-reducing bacteria. In: Zehnder AJB, ed. *Biology of anaerobic microorganisms.* New York: J Wiley, 1988: 469–585.
7 Gibson GR, Cummings JH, MacFarlane GT. Factors affecting hydrogen uptake by bacteria growing in the human large intestine. In: Bélaich J-P, Bruschi M, Garcia J-L, eds. *Microbiology and biochemistry of strict anaerobes involved in inter species hydrogen transfer.* New York: Plenum Press, 1990: 191–202.
8 Gibson GR, MacFarlane GT, Cummings JH. Sulphate reducing bacteria and hydrogen metabolism in the human large intestine. *Gut* 1993; **34:** 437–9.
9 Strocchi A, Ellis GJ, Furne JK, Levitt MD. Study of constancy of hydrogen-consuming flora of human colon. *Dig Dis Sci* 1994; **39:** 494–7.
10 Gibson GR, Cummings JF, MacFarlane GT. Competition for hydrogen between sulphate reducing bacteria and methanogenic bacteria from the human large intestine. *J Appl Bacteriol* 1988; **65:** 241–7.
11 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.
12 Strocchi A, Furne J, Ellis C, Levitt MD. Methanogens outcompete sulphate reducing bacteria for H_2 in the human colon. *Gut* 1994; **35:** 1098–101.
13 Florin T, Neale G, Gibson GR, Christl SU, Cummings JH. Metabolism of dietary sulphate: absorption and excretion in humans. *Gut* 1991; **32:** 766–73.
14 Friedberg F, Tarver H, Greenberg DM. The distribution pattern of sulfur-labeled methionine in the protein and the free amino acid fraction of tissues after intravenous administration. *J Biol Chem* 1948; **173:** 355–61.

15 Jennings MA, Florey HW. Autoradiographic observations on the mucous cells of the stomach and intestine. *Q J Exp Physiol* 1956; **41:** 131–52.

16 Garcia RAG, Stipanuk MH. The splanchnic organs, liver and kidney have unique roles in the metabolism of sulfur amino acids and their metabolism in rats. *J Nutr* 1992; **122:** 1693–701.

17 Dahm LJ, Jones DP. Secretion of cysteine and glutathione from mucosa to lumen in rat small intestine. *Am J Physiol* 1994; **267:** G292–300.

18 Ohkusa T. Production of experimental ulcerative colitis in hamsters by dextran sulfate sodium and change in intestinal microflora. *Jpn J Gastroenterol* 1985; **82:** 1337–47.

19 Ishioka T, Kuwabara N, Oohashi Y, Wakabayashi K. Induction of colorectal tumours in rats by sulfated polysaccharides. *CRC Crit Rev Toxicol* 1986; **17:** 215–44.

20 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.

21 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:** 623–7.

22 Florin THJ, Gibson GR, Neale G, Cummings JH. A role for sulphate reducing bacteria in ulcerative colitis. *Gastroenterology* 1990; **98:** A170.

23 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.

24 Weiseger RA, Pinkus LM, Jakoby WB. Thiol S-methyltransferase: suggested role in detoxication of intestinal hydrogen sulfide. *Biochem Pharmacol* 1980; **29:** 2885–7.

25 Pacifici GM, Romiti P, Santerini S, Giuliani L. S-methyltransferases in human intestine: differential distribution of the microsomal methyltransferase and cytosolic thiopurine methyltransferase along the human bowel. *Xenobiotica* 1993; **23:** 671–9.

26 Eloranta TO. Tissue distribution of S-adenosyl methionine and S-adenosylhomocysteine in the rat. *Biochem J* 1977; **166:** 521–9.

27 Roediger WEW. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa. *Gut* 1980; **21:** 793–8.

28 Roediger WEW. Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology* 1982; **83:** 424–9.

29 Bergmeyer HU, Bergmeyer J, Grassl M. *Methods of enzymatic analysis.* 3rd ed. Weinheim: Verlag Chemie, 1984; vol VI, 582–8, vol VIII, 61–9.

30 Babidge W, Millard S, Roediger W. Effect of sodium hydrogen sulfide and butyrate on acetylation of 5-aminosalicylic acid in isolated rat colonocytes. *Med Sci Res* 1994; **22:** 219–20.

31 Ahlman B, Leijonmarck C-E, Lind C, Vinnars E, Wernerman J. Free amino acids in biopsy specimens from the human colonic mucosa. *J Surg Res* 1993; **55:** 647–53.

32 Lund J, Stjernström H, Vinnars E, Jorfeldt L, Bergholm U, Wiklund L. The influence of abdominal surgery on the splanchnic exchange of amino acids. *Acta Chir Scan* 1986; **152:** 191–7.

33 Darragh AJ, Cranwell PD, Moughan PJ. Absorption of lysine and methionine from the proximal colon of the piglet. *Br J Nutr* 1994; **71:** 739–52.

34 Martensson J, Jain A, Meister A. Glutathione is required for intestinal function. *Proc Natl Acad Sci* 1990; **87:** 1715–9.

35 Kelly FJ. Glutathione content of the small intestine: regulation and function. *Br J Nutr* 1993; **69:** 589–96.

36 Bray TM, Taylor CG. Enhancement of tissue glutathione for antioxidant and immune functions in malnutrition. *Biochem Pharmacol* 1994; **47:** 2113–23.

37 Creveling CR, Thakker DR. O-, N-, and S-methyltransferases. *Handbook Exp Pharmacol* 1993; **112:** 189–216.

38 Rao AM, Drake MR, Stipanuk MH. Role of the transsulfuration pathway and of γ cystathionase activity in the formation of cysteine and sulfate from methionine in rat hepatocytes. *J Nutr* 1990; **120:** 837–45.

39 Cooper AJL. Biochemistry of sulfur-containing amino acids. *Ann Rev Biochem* 1983; **52:** 187–222.

40 Friedel HA, Goa KL, Benfield P. S-adenosyl-L-methionine. A review of its pharmacological properties and therapeutic potential in liver dysfunction and affective disorders in relation to its physiological role in cell metabolism. *Drugs* 1989; **38:** 389–416.

41 Di Padova C. Adenosylmethionine in the treatment of osteoarthritis. Review of the clinical studies. *Am J Med* 1987; **83** (suppl 5A): 60–5.

42 Chawla RK, Bonkovsky HL, Galambos JT. Biochemistry and pharmacology of S-adenosyl-L-methionine and rationale for its use in liver disease. *Drugs* 1990; **40** (suppl 3): 98–110.

43 Salim AS. Role of sulphydryl-containing agents in the management of recurrent attacks of ulcerative colitis. A new approach. *Pharmacology* 1992; **45:** 307–18.

44 Fields J, Keshavarzian A, Eiznhamer D, Frommel T, Winship D, Holmes E. Low levels of blood and colonic mucosal glutathione in ulcerative colitis. *Gastroenterology* 1994; **106:** A248.

45 Brown MD, Dudeja PK, Brasitus A. S-adenosyl-L-methionine modulates $Na^+ + K^+$-ATPase activity in rat colonic basolateral membranes. *Biochem J* 1988; **251:** 215–22.

46 Gibson GR, MacFarlane S, MacFarlane GT. Metabolic interactions involving sulphate-reducing and methanogenic bacteria in the human large intestine. *FEMS Microbiol Ecol* 1993; **12:** 117–25.

47 Gibson GR, MacFarlane GT, Cummings JH. Occurrence of sulphate-reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut. *J Appl Bacteriol* 1988; **65:** 103–11.

48 Roediger WEW. Failed redox control as a cause of ulcerative colitis. In: Monteiro E, Veloso FT, eds. *Inflammatory bowel diseases.* Kluwer, Dordrecht: 1995: 61–71.

49 Roediger WEW, Duncan A. 5-ASA decreases colonic sulphide formation: implications for ulcerative colitis. *Med Sci Res* 1996; **24:** 27–9.

50 Pitcher MCL, Beatty ER, Cummings JH. Salicylates inhibit bacterial sulphide production within the colonic lumen in ulcerative colitis. *Gut* 1995; **37:** A15.

51 Kameyana J, Narui H, Inui M, Sato T. Energy level in large intestinal mucosa in patients with ulcerative colitis. *Tohuku J Exp Med* 1984; **143:** 253–4.