

# Fecal Hydrogen Production and Consumption Measurements

## Response to Daily Lactose Ingestion by Lactose Maldigesters

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The alteration of hydrogen ( $H_2$ ) metabolism, which accounts for the large decrease in breath  $H_2$  excretion following prolonged ingestion of malabsorbed carbohydrate (lactulose, lactose in lactose maldigesters) was studied in six lactose-maldigesting adults. Metabolic inhibitors of the three main  $H_2$ -consuming reactions (methanogenesis, sulfate reduction, and acetogenesis) were used to independently measure  $H_2$  production and consumption in fecal samples obtained after 10 days of either dextrose or lactose feeding. Absolute fecal  $H_2$  production (net of production minus consumption) after 3 hr of incubation with lactose was approximately threefold lower after lactose adaptation ( $242 \pm 54 \mu\text{l}$ ) compared to dextrose adaptation ( $680 \pm 79 \mu\text{l}$ ,  $P = 0.006$ ). Fecal  $H_2$  consumption was not affected by either feeding period. We conclude that decreased absolute  $H_2$  production, rather than increased  $H_2$  consumption, is responsible for the decrease in breath  $H_2$  observed with lactose feeding.

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**KEY WORDS:** colonic adaptation; hydrogen production; lactose maldigestion.

Fermentation of malabsorbed carbohydrate by the colonic bacteria results in the production of short-chain fatty acids and gases [ $H_2$ , carbon dioxide ( $CO_2$ ) and methane ( $CH_4$ )]. The stoichiometry of a major fermentation reaction indicates that about 13,600 ml of hydrogen should be produced from the approximately 40 g of carbohydrate that has been estimated

to reach the colon each day (1, 2). The actual release of such volumes of hydrogen obviously would have important implications for intestinal distension, rectal gas problems, and explosions. Fortunately, the vast majority of this  $H_2$  is consumed by other colonic bacteria and only a minor fraction, the net of absolute production minus consumption, actually escapes from the feces and is available for excretion via the anus or the lungs.

A number of previous studies have shown that the breath  $H_2$  response to ingestion of a malabsorbed sugar [lactulose (3–5) or lactose (6, 7)] is markedly reduced following ingestion of the sugars for one to two weeks. The mechanism for the decrease in breath hydrogen following colonic adaptation to carbohydrate is unclear. Some authors have hypothesized that adaptation results in greater fermentation by organisms that can metabolize lactose/lactulose without the evolution of  $H_2$ , such as bifidobacteria or other lactic acid bacteria (3, 8). It is also possible that bacterial

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TABLE 1. TREATMENT SCHEME FOR MEASUREMENT OF FECAL HYDROGEN PRODUCTION AND CONSUMPTION

<i>Treatment</i>	<i>Fecal homogenate volume (ml)</i>	<i>Headspace gas volume (ml)</i>	<i>Headspace gas composition</i>	<i>Inhibitors of H<sub>2</sub> consumption*</i>	<i>Addition of lactose†</i>	<i>Measurement</i>
1	5	25	10% CO <sub>2</sub> 90% argon	N	N	Net H <sub>2</sub> production, no substrate
2	5	25	10% CO <sub>2</sub> 90% argon	Y	Y	Absolute H <sub>2</sub> production from lactose
3	5	25	10% CO <sub>2</sub> 90% argon	N	Y	Net H <sub>2</sub> production from lactose
4	5	25	10% H <sub>2</sub>	N	N	H <sub>2</sub> consumption

\* Inhibitors added were 20 mmol/liter homogenate BES (inhibits methanogenesis), 20 mmol/liter Mo (inhibits sulfate reduction), and 50 µmol/liter chloroform (inhibits acetogenesis).

† Lactose (5.5 mmol/liter homogenate) was added as fermentable substrate in treatments 2 and 3.

consumption of H<sub>2</sub> is up-regulated. To distinguish between these two possibilities, it is necessary to independently measure either absolute H<sub>2</sub> production or H<sub>2</sub> consumption. Recently, we described a technique to measure absolute H<sub>2</sub> production via the use of metabolic inhibitors of the major H<sub>2</sub>-catabolizing reactions utilized by human colonic bacteria (9). In the present study, this technique was employed to determine if decreased absolute H<sub>2</sub> production and/or increased H<sub>2</sub> consumption accounts for the decreased H<sub>2</sub> excretion observed after colonic adaptation to ingestion of nonabsorbed carbohydrate.

## MATERIALS AND METHODS

**Subjects.** Six free-living subjects (five men, one woman;  $\bar{X}$  = 68 kg and 35 years of age) were studied. All subjects were in good health and had not used antibiotics in the preceding two months. Subjects were identified as lactose maldigesters based on a rise in breath hydrogen of >20 parts per million (0.9 µmol H<sub>2</sub>/liter of air) following an aqueous lactose dose (0.35 g/kg body weight) administered after an overnight fast (10). One subject was a large methane producer (breath CH<sub>4</sub> > 20 ppm above the atmospheric concentration), while the breath methane of the remainder of the subjects was <1 ppm above atmospheric. Informed consent was obtained and the protocol for the study was approved by the Institutional Review Board Human Subjects Committee at the University of Minnesota.

**Experimental Design.** This study was a 22-day blinded, randomized feeding trial. Subjects were instructed to avoid all products high in lactose (milk, ice cream, yogurt) but otherwise were allowed to eat their normal diet. This diet was supplemented with either lactose (Land O'Lakes, Arden Hills, Minnesota) or dextrose (control) for days 1–11 and crossed over to the other treatment for days 12–22. The initial dose of sugar was 0.6 g/kg body wt/day and was increased by 0.2 g/kg increments every other day, up to a maximum of 1.0 g/kg/day by the end of the feeding period. The sugar supplements were ingested in three equal doses in 250 ml of tap water with breakfast, lunch, and dinner. At

the end of each feeding period (days 11 and 22), subjects collected a single fecal sample for analysis.

**Fecal Sample Collection.** Fecal samples were collected in the morning on days 11 and 22. Subjects were provided with a stool collection apparatus (Commode Specimen Collection System, Sage, Inc., Crystal Lake, Illinois) and a polyethylene bag was used to line the inside of the container. Subjects defecated into the bag and were instructed to immediately squeeze residual air out of the bag before sealing it tightly with a rubber band. Fecal samples were transported to the laboratory within 1 hr of defecation and were processed within 3–4 hr.

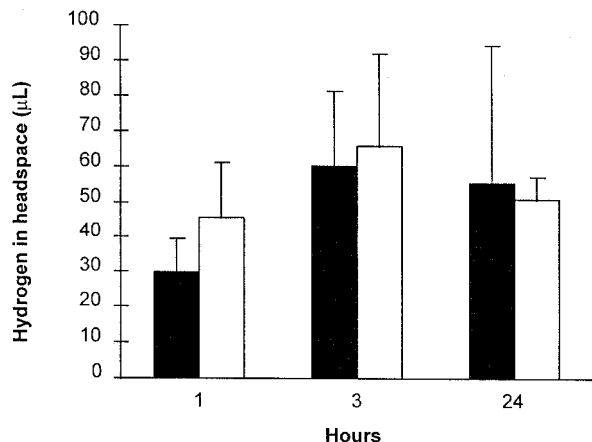
**Measurement of Fecal Hydrogen Production and Consumption.** A weighed sample of feces was placed in a blender vessel fitted with a gas-tight lid. After exhaustively flushing the vessel with argon, deoxygenated buffer (0.2 M PO<sub>4</sub> buffered saline), which was warmed to 37°C, was added (1:20 w/v). The feces were then mixed for the minimal period (seconds) required to produce a smooth homogenate. A 5-ml aliquot of homogenate was anaerobically aspirated into each of eight plastic 60-ml syringes that had been previously purged with argon. The syringes were sealed with stopcocks, and four different measurements of H<sub>2</sub> metabolism were then carried out in duplicate: (1) net H<sub>2</sub> production from substrates endogenous to feces, (2) absolute H<sub>2</sub> production from lactose fermentation, (3) net H<sub>2</sub> production from lactose fermentation, and (4) H<sub>2</sub> consumption. The headspace gases and the additions to the homogenate for each of these four measurements are shown in Table 1.

The four sets of syringes were incubated in an orbital shaker at 37°C. At time 0 (syringe containing 10% H<sub>2</sub>) and hours 1, 3, and 24 (all syringes), a 0.5-ml gas sample was removed for analysis using a 0.5-ml gas-tight syringe.

Gas samples were analyzed for H<sub>2</sub> and CH<sub>4</sub> content via a gas chromatograph equipped with a molecular sieve column and a reduction detector for H<sub>2</sub> and a hydrogen flame detector for CH<sub>4</sub> (10). The coefficient of variation for the duplicate measurements averaged 8.5%.

After the last gas sample was drawn at 24 hr of incubation, the pH of the fecal homogenates was obtained using a pH meter (Fisher Scientific, Pittsburgh, Pennsylvania).

**Calculations and Statistics.** Data are expressed as the

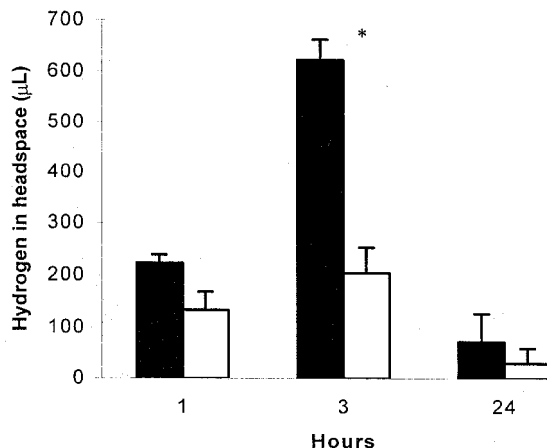


**Fig 1.** Net fecal  $H_2$  production without additional substrate. Solid bars are after dextrose adaptation; open bars are after lactose adaptation. Bars represent the mean  $\pm$  SEM ( $N = 6$  for hours 1 and 3,  $N = 5$  for 24 hr).

mean  $\pm$  SEM ( $N = 6$ ). The methane-producing subject was included in the analysis after calculation of hydrogen equivalents, using the stoichiometry of 4 mol  $H_2$  consumed to yield 1 mol  $CH_4$  (2). However, data were unavailable for the 24-hr time point for this subject due to a laboratory error, and thus,  $N = 5$  for the 24-hr production calculations. Analysis of variance (ANOVA) was used to determine differences in  $H_2$  production and consumption among the four types of measurements, the three time points, and between the lactose and dextrose feeding periods. Where significant differences were found, Tukey's HSD test was used for multiple comparisons (11, 12).

## RESULTS

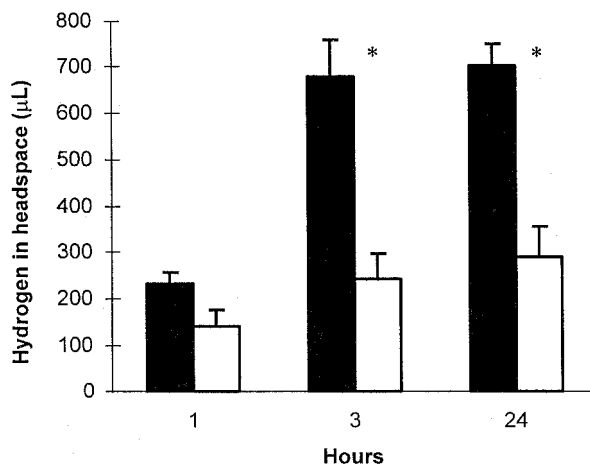
Net  $H_2$  production by the fecal samples incubated without additional fermentable substrate is shown in Figure 1. There were no significant differences between the dextrose- and lactose-adapted periods at any time point. Figure 2 shows the net  $H_2$  production resulting from the fermentation of lactose added to the homogenate (calculated from the difference between the  $H_2$  production in the homogenate containing added lactose and that in the control syringe). Hydrogen production at 3 hr was significantly reduced ( $P = 0.001$ ) by lactose adaptation ( $205 \pm 50 \mu L H_2$ ) compared to dextrose adaptation ( $623 \pm 39 \mu L H_2$ ). The quantity of  $H_2$  in the syringes declined sharply between 3 and 24 hr as the rate of  $H_2$  consumption exceeded the rate of production. By 24 hr, net  $H_2$  production was not different between the lactose and dextrose periods. Figure 3 shows the absolute  $H_2$  production by the fecal samples. There was no significant difference in  $H_2$  production at the 1-hr time point. However, at 3 hr absolute  $H_2$  production after the lactose feeding period ( $242 \pm 54 \mu L$ ,



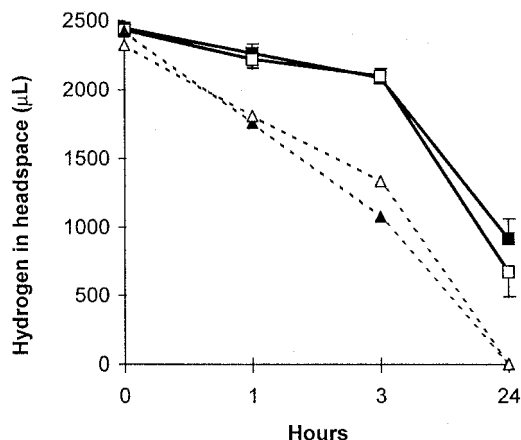
**Fig 2.** Net fecal  $H_2$  production from lactose added *in vitro*. Solid bars are after dextrose adaptation; open bars are after lactose adaptation. Bars represent the mean  $\pm$  SEM ( $N = 6$  for hours 1 and 3,  $N = 5$  for 24 hr). \*  $P = 0.001$ .

$P = 0.006$ ) was only about one third that after the dextrose period ( $680 \pm 79 \mu L$ ,  $P = 0.006$ ) and a similar difference was maintained after 24 hr of incubation ( $289 \pm 68 \mu L$  vs  $703 \pm 48 \mu L$ ,  $P = 0.003$ ).

Fecal  $H_2$  consumption is shown in Figure 4. There was no significant difference in the rate of  $H_2$  consumption by feces collected after the dextrose versus lactose feeding periods. The  $CH_4$ -producing feces consumed  $H_2$  far more rapidly ( $>99\%$  consumed in 24 hr) than did the non- $CH_4$ -producing feces (approximately 70% consumed in 24 hr), but there was no difference in the  $H_2$  consumption rate after lactose or dextrose feeding in the methanogenic feces. Meth-



**Fig 3.** Absolute fecal  $H_2$  production from lactose added *in vitro*. Solid bars are after dextrose adaptation; open bars are after lactose adaptation. Bars represent the mean  $\pm$  SEM ( $N = 6$  for hours 1 and 3,  $N = 5$  for 24 hr). \*  $P = 0.006$ ; \*\*  $P = 0.003$ .



**Fig 4.** Fecal H<sub>2</sub> consumption from headspace containing 10% (2500 µl/25 ml) hydrogen. Solid lines are means of data for methane nonproducers ( $N = 5$ ); dashed lines are for the methane producer ( $N = 1$ ). Open symbols are after dextrose adaptation; closed symbols are after lactose adaptation. Values for methane nonproducers are the mean  $\pm$  SEM.

ane production was always negligible in the feces of the non-CH<sub>4</sub> producing subjects.

The pH of the fecal homogenates ranged between 7.0 and 6.5 after 24 hr of incubation. However, pH was significantly lower ( $P = 0.002$ ) in the homogenates to which lactose had been added (pH  $6.69 \pm 0.02$ ) versus those to which no lactose had been added (pH  $6.84 \pm 0.92$ ).

## DISCUSSION

The goal of this study was to identify the alteration of fecal H<sub>2</sub> metabolism that accounts for the decreased H<sub>2</sub> excretion observed in individuals who have ingested nonabsorbable carbohydrates (eg, lactulose, lactose in lactose maldigesters) for one to two weeks (3–7). Clinical measurements of H<sub>2</sub> production, such as breath H<sub>2</sub> excretion, assess the net of absolute H<sub>2</sub> production minus consumption of this gas. While bacterial production of H<sub>2</sub> has received extensive study, the importance of fecal H<sub>2</sub> consumption has only been recently recognized. The bulk of H<sub>2</sub> consumption by human feces appears to be accounted for by three reactions—methanogenesis, sulfate reduction, and acetogenesis. The relative importance of the mechanisms appears to have a geographical or methodological variability. British studies suggest that virtually all H<sub>2</sub> catabolism can be accounted for by sulfate-reducing or methanogenic bacteria (13). In contrast, our study of H<sub>2</sub> consumption by feces obtained from 14 subjects in Minneapolis indicated that methanogenesis, sulfate reduction, and other reactions, presumably acetogenesis, were

predominant in 35%, 30%, and 35% of subjects, respectively (9).

There is increasing evidence that abnormalities of H<sub>2</sub> consumption may play a role in colonic disease. The most clear-cut example is the recent demonstration that a lack of hydrogen consumption accounts for the enormous hydrogen excretion and presumably the intestinal cysts of patients with pneumatosis cystoides intestinalis (14). In addition, sulfate-reducing bacteria utilize hydrogen to reduce sulfate to sulfide, and it has been suggested that the highly toxic hydrogen sulfide produced might play an etiological role in ulcerative colitis (15). Lastly, while not yet clearly demonstrated, it appears likely that an imbalance between H<sub>2</sub> production and H<sub>2</sub> consumption plays an important role in excessive flatulence (16, 17).

To identify the role of H<sub>2</sub> production versus H<sub>2</sub> consumption in the genesis of an unusual net production of this gas, it is necessary to measure either absolute production or consumption or both. The conventional means of determining the rates of the simultaneously ongoing production and consumption reactions utilizes the disappearance rate of a different isotope of the compound whose production is being determined. However, this approach is not feasible in the case of H<sub>2</sub> metabolism because of the extremely rapid exchange of hydrogen atoms in H<sub>2</sub> with those in fecal water (18), and alternative methodologies must be employed.

When the supply of fermentable substrate is limited, the rate of H<sub>2</sub> consumption at high H<sub>2</sub> tensions far exceeds the rate of absolute H<sub>2</sub> production. Thus, the disappearance of H<sub>2</sub> from the headspace over fecal samples incubated without added substrate provides a measure of the H<sub>2</sub>-consuming ability of the homogenate. We have studied two different methods of measuring absolute H<sub>2</sub> production. Since hydrogen consumption is proportional to H<sub>2</sub> tension in the homogenate, H<sub>2</sub> consumption can be minimized by maintenance of an extremely low fecal H<sub>2</sub> tension. This has been achieved by incubating thin films of fecal homogenate underneath a very large gas space (19). A simpler approach, that of the present study, involves the use of metabolic inhibitors of the H<sub>2</sub>-consuming reactions. Methanogenesis, sulfate reduction, and acetogenesis are specifically inhibited by 2-bromoethanesulfonic acid, sodium molybdate, and chloroform, respectively (20–22). Thus, the H<sub>2</sub> released by a homogenate containing these three inhibitors provides a measurement of absolute H<sub>2</sub> production.

In the present study fecal samples were collected

from lactose-maldigesting subjects who had ingested large quantities of either lactose or dextrose for 10-day periods. The absolute production and consumption rates of  $H_2$  by fecal homogenates were then determined using the above-described techniques. As shown in Figure 3, the absolute  $H_2$  production rate during lactose fermentation was only about one third as rapid ( $P = 0.006$ ) in feces collected after the lactose versus the dextrose feeding period. In contrast, there was no significant difference between the  $H_2$  consumption rates observed after the two feeding periods. Thus, we conclude that the decreased  $H_2$  response to lactose after lactose adaptation results from a decreased absolute production of  $H_2$ , whereas  $H_2$  consumption is not altered. This finding has important implications for the development of stoichiometric equations that predict the amounts of SCFA and  $H_2$  that could be generated from a given amount of fermentable carbohydrate. The existing equations do not take into account the effect of adaptation on  $H_2$  production. It has been demonstrated that adaptation to lactulose (3, 4) results in a shift toward the generation of acetic and lactic acids, rather than  $H_2$ , as end products of fermentation.

While this study does not shed light on the mechanism by which lactose feeding reduces absolute  $H_2$  production, previous work has shown marked increases in fecal  $\beta$ -galactosidase (3, 4, 6, 7) and reduced fecal hexose excretion (3, 4) after lactose or lactulose adaptation. Induction of increased levels of  $\beta$ -galactosidase in the existing flora would not be expected to reduce  $H_2$  production. Rather, it seems necessary to postulate that lactose stimulates the proliferation of bacterial species such as bifidobacteria or other lactic acid bacteria that carry out non- $H_2$ -producing, lactose-fermentation reactions (23). In support of this concept is the demonstration of Terada et al (24) that chronic administration of lactulose (3 g/day for 14 days) resulted in a fivefold increase in fecal bifidobacteria counts and a 2 log decrease in lecithinase-positive clostridia (which are  $H_2$  producers). Bifidobacteria also have been shown to inhibit other  $H_2$  producing bacteria, such as *Escherichia coli* (25, 26). Perman et al have proposed that the fermentation of malabsorbed lactose reduces fecal pH, and this low pH inhibits further lactose catabolism (5). This clearly cannot be the explanation for our findings, since the pH of the fecal homogenates was maintained between 7 and 6.5 in all instances.

Given the minimal symptoms that result from lactose malabsorption in the adapted colon (6, 7) and

the many claims that high bifidobacteria populations are beneficial to health (23), it seems possible that a potential approach to lactose maldigestion is the regular ingestion of dairy products rather than lactose avoidance or the use of lactose digestive aids.

## REFERENCES

1. Wolin MJ: Interactions between  $H_2$ -producing species. In *Microbial Formation and Utilization of Gases*. HG Schlegel, N Pfennig (eds). Gottingen, Germany, Goltze Press, 1976, p 141
2. Levitt MD, Gibson GR, Christl SU: Gas metabolism in the large intestine. In *Human Colonic Bacteria: Role in Nutrition, Physiology, and Pathology*. GR Gibson, GT Macfarlane (eds). Boca Raton, Florida, CRC Press, 1995, p 136
3. Florent C, Flourie B, Leblond A, Rautureau M, Bernier J-J, Rambaud J-C: Influence of chronic lactulose ingestion on the colonic metabolism of lactulose in man (an *in vivo* study). *J Clin Invest* 75:608–613, 1985
4. Flourie B, Briet F, Florent C, Pellier P, Maurel M, Rambaud J-C: Can diarrhea induced by lactulose be reduced by prolonged ingestion of lactulose? *Am J Clin Nutr* 58:369–375, 1993
5. Perman J, Modler S, Olson AC: Role of pH in production of hydrogen from carbohydrates by colonic bacterial flora: Studies *in vivo* and *in vitro*. *J Clin Invest* 67:643–650, 1981
6. Hertzler S, Savaiano DA: Daily lactose feeding improves lactose tolerance by enhancing colonic fermentation. *Gastroenterology* 108:A328, 1995 (abstract)
7. Arrigoni E, Pochart P, Flourie B, Marteau P, Franchisseur C, Rambaud J-C: Does a prolonged lactose ingestion induce clinical and colonic metabolism adaptations in lactose intolerant subjects? *Gastroenterology* 102:A197, 1992 (abstract)
8. Hill MJ: Bacterial adaptation to lactase deficiency. In *Milk Intolerances and Rejection*. J Delmont (ed). New York, Karger, 1983, pp 22–26
9. Strocchi A, Ellis CJ, Levitt MD: Use of metabolic inhibitors to study  $H_2$  consumption by human feces: Evidence for a pathway other than methanogenesis and sulfate reduction. *J Lab Clin Med* 121:320–327, 1993
10. Strocchi A, Corraza G, Ellis CJ, Gasbarrini G, Levitt MD: Detection of malabsorption of low doses of carbohydrate: Accuracy of various breath  $H_2$  criteria. *Gastroenterology* 105:1404–1410, 1993
11. Systat, Inc. SYSTAT for the Macintosh, version 5.2. Evanston, Illinois, Systat, 1992
12. Montgomery DC: *Design and Analysis of Experiments*, 3rd ed. New York, John Wiley & Sons, 1991, pp. 129–130
13. Gibson GR, Cummings JH, Macfarlane GT, Allison C, Segal I, Vorster HH, Walker ARP: Alternative pathways for hydrogen disposal during fermentation in the colon. *Gut* 31:679–683, 1990
14. Christl SU, Gibson GR, Murgatroyd PR, Scheppach W, Cummings JH: Impaired hydrogen metabolism in pneumatosis cystoides intestinalis. *Gastroenterology* 104:392–397, 1993
15. Roediger WEW, Duncan A, Kapaniris O, Millard S: Reducing sulfur compounds of the colon impair colonocyte nutrition: Implications for ulcerative colitis. *Gastroenterology* 104:802–809, 1993

16. Price KR, Lewis J, Wyatt GM, Fenwick GR: Flatulence—causes, relation to diet and remedies. *Die Nahrung* 32:609–626, 1988
17. Levitt MD, Lasser RB, Schwartz JS, Bond JH: Studies of a flatulent patient. *N Engl J Med* 295:260–262, 1976
18. Strocchi A, Klein PD, Wong W, Levitt MD: Use of deuterium to study H<sub>2</sub> consumption by human feces: Evidence for rapid deuterium–hydrogen exchange. *Biochem Arch* 8:33–38, 1992
19. Strocchi A, Levitt MD: Factors affecting hydrogen production and consumption by human fecal flora: The critical roles of hydrogen tension and methanogenesis. *J Clin Invest* 89:1304–1311, 1992
20. Smith MR: Reversal of 2-bromoethanesulfonic acid inhibition of methanogenesis in *Methanosarcina* sp. *J Bacteriol* 156:516–523, 1983
21. Taylor BF, Oremland RS: Depletion of adenosine triphosphate in *Desulfovibrio* by oxyanions of group VI elements. *Curr Microbiol* 3:101–103, 1979
22. Prins RA, Lankhorst A: Synthesis of acetate from CO<sub>2</sub> in the cecum of some rodents. *FEMS Microbiol Lett* 1:255–258, 1977
23. Ballongue J: Bifidobacteria and probiotic action. In Lactic Acid Bacteria. S Selminen, A von Wright (eds). New York, Marcel Dekker, 1993, pp 369–371
24. Terada A, Hara H, Kataoka M, Mitsuoka T: Effect of lactulose on the composition and metabolic activity of the human fecal flora. *Microb Ecol Health Dis* 5:43–50, 1992
25. Gibson GR, Wang X: Regulatory effects of bifidobacteria on the growth of other colonic bacteria. *J Appl Bacteriol* 77:412–420, 1994
26. Gibson GR, Beatty ER, Wang X, Cummings JH: Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* 108:975–982, 1995