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Production, Metabolism, and Excretion of Hydrogen in the Large Intestine

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Hydrogen is produced during fermentation in the large intestine and may be excreted in breath and flatus or further metabolized by the flora. However, there is little information about total H_2 excretion from different substrates or the extent to which it is metabolized in the colon. We have therefore measured total H_2 and methane excretion in 10 healthy subjects using a whole body calorimeter. Breath gases were measured simultaneously with total excretion in response to lactulose, pectin, and banana starch. Metabolic activities of the predominant H_2 consuming anaerobes (methanogenic, sulfate reducing, and acetogenic bacteria) were measured in fecal samples. Total H_2 excretion on a starch and fiber-free diet was 35 ± 6.1 mL/24 h ± SEM. H_2 from 7.5 g, 15 g, and 22.5 g lactulose was 88.1 ± 22.4 mL, 227.0 ± 60.7 mL, and 321.8 ± 79.2 mL. Four of the subjects also excreted CH_4, which was 51.3 ± 5.5 mL, 97.3 ± 18.4 mL, and 157.5 ± 36.3 mL for the respective lactulose doses. H_2 excretion was less in methanogenic subjects (7.9 mL/g lactulose) than in nonmethanogenic (17.3 mL/g), but total H_2 excreted as, hydrogen + methane, was 34.9 mL/g. H_2 from pectin (20 g) was 14.1% ± 3.2% and from starch (22.2 g) 38.6% ± 9.2% of an equivalent lactulose dose. Sixty-five percent of total H_2 and CH_4 was expired in breath at total excretion rates up to 200 mL/24 h. Over this the proportion decreased to 25% with an overall average of 58%. Only subjects with CH_4 excretion in vivo showed methanogenesis in feces, whereas nonmethanogenic subjects showed high sulfate-reducing activity in feces (58.7 ± 5.6 nmol $^{35}SO_4$ reduced · h^{-1} · g^{-1} wet wt vs. 7.9 ± 2.0 nmol · h^{-1} · g^{-1} in methanogens). Acetogenesis rates were very low in both groups. It was concluded that H_2 excretion varies with different substrates. The proportion of H_2 that is exhaled in breath is higher than currently accepted and varies with total excretion rate. Substantial amounts of H_2 are consumed by methanogenic and sulfate-reducing bacteria.

Fermentation, the breakdown of carbohydrate by anaerobic bacteria in the large intestine of humans is a major function of the colon.[1] Through fermentation, the host salvages energy from malabsorbed carbohydrate, principally starch, nonstarch polysaccharides (dietary fiber), and sugars.[2–4] Quantitative estimates of fermentation are essential to an understanding of colonic physiology and metabolism in man but few data on the amount of material fermented and products formed are available.

Hydrogen is an essential product of fermentation but, in contrast to short chain fatty acids, is not metabolized by mammalian cells. It is expelled either as flatus or absorbed and expired in breath.[5] Breath hydrogen has been used in many studies to estimate the amount of substrate being fermented.[6–8] Such studies assume that a constant proportion of H_2 generated is excreted in breath, and that there is the same amount of H_2 produced from equal amounts of any substrate fermented. In practice, the results of breath studies show great variability and considerable discrepancies when compared with more direct measurements of carbohydrate absorption by ileal intubation[9] and in ileostomy studies.[10,11] These assumptions may therefore be incorrect.

H_2 is formed as a means of disposing of reducing equivalents from the anaerobic colonic environment,[12] but a high partial pressure of H_2 in anaerobic ecosystems inhibits fermentation efficiency.[13] Therefore, in the rumen most H_2 is used to reduce CO_2 to CH_4. In humans, only 30%–40% of European populations are methanogenic.[14,15] Nonmethanogenic individuals carry bacteria that can dispose of H_2 and other electron donors by reducing sulfate to sulfide.[16] Some fecal bacteria may also form acetate from CO_2 and H_2.[17]

To quantitate H_2 excretion from different substrates and evaluate the importance of these different metabolic pathways, a whole body calorimeter was used to measure total excretion of H_2 and CH_4 over a 36-hour period in response to lactulose, pectin, and starch. Breath samples were collected at the same time, and the influence of methanogenesis, sulfate reduction, and acetogenesis on production and excretion of hydrogen were examined by in vitro studies of bacterial metabolism.

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Materials and Methods

Subjects

Ten healthy subjects (6 male, 4 female, age 18–46 years) who had no history of gastrointestinal disease or recent use of antibiotics took part in the study. The subjects ate a polysaccharide free basal diet and spent 4–6 36-hour periods in a whole body calorimeter while test meals of lactulose in three doses, pectin, and starch were given. Total and breath gas measurements were made, and feces were collected for bacteriology. Informed consent was obtained, and the protocol was approved by the ethical committee of the MRC Dunn Nutrition Unit.

Diet and Test Carbohydrates

A polysaccharide-free basal diet was given to minimize background gas production. This contained egg, bacon, milk, tea, coffee, sugar, prawns, mayonnaise, meat, cheese, jelly, and cream. The nutrient composition was carbohydrate, 61 g; protein, 107.5 g; fat, 94.2 g; and energy, 6.3 mJ. Between studies, the volunteers were allowed a free diet. Lactulose (Duphalac, Duphar Ltd., Southampton, England) was given in doses of 7.5 g, 15 g, and 22.5 g each diluted to 250 mL with water. Twenty grams of Pectin (HP Bulmer, Hereford, England) was mixed with 400 mL of water. White bread was freshly baked using the following ingredients: 500 g of strong wheat flour, 12 g of lard, 10 g of salt, 7 g of dried yeast, and 400 mL of water. Two hundred twelve grams equivalent to 100 g of starch were given. Bananas were purchased locally and given when not fully ripe, providing a starch content of 20%–40% of dry weight.[18] The amount given varied between 250 g and 350 g and was decided by signs of ripeness based on previous analyses.[18] An aliquot of each fruit was immediately frozen on dry ice and then freeze dried for later starch analysis.

Study Protocol

On day 1, the subjects began the basal diet, and at 7 PM calorimeters were entered. At 8 AM on day 2, either 0 g, 7.5 g, 15 g, or 22.5 g of Lactulose were given in random order. All four doses were tested in each subject, and 4 subjects repeated the 15-g dose to test for reproducibility. The pectin and banana meal was given to 5 volunteers each. There was no other breakfast on day 2 apart from coffee but the basal diet was fed for the rest of the day. At 8 AM on day 3, calorimeters were left. Calorimeter, gas, and end-expiratory breath samples were collected every half hour from 8 AM to 11 PM and then hourly through the night for calorimeter gases only. With white bread (5 subjects) only breath gas was collected.

Fecal specimens for bacteriology were obtained from each subject at the end of two-calorimeter runs and total collections performed for 5 days after the banana meal to measure starch excretion.

Calorimeter

Two whole-body calorimeters (10.5 m^3 and 11.5 m^3) were used.[19] These consisted of an airtight chamber equipped with an air lock. Fresh air was passed at a flow rate of 100 L/min. Gas samples were collected from ingoing and outgoing air. Gas concentrations were corrected to standard temperature and pressure (STP) and production rates of H_2, CH_4, and CO_2 calculated using this formula[20]:

$$Rg = [Fi \times Ki \times (fGo - fGi)] + (V \times Ko \times d/dt \times fGo)$$

where Rg = gas production rate, F = flow rate, V = calorimeter volume, fG = fractional gas concentration, i = ingoing, o = outgoing. K = conversion factors to STP:

$$Ki = \sqrt{P \times 258.78/760 \times Ti}$$

$$\text{and} \quad Ko = 273 \times P/760 \times To$$

where P = pressure (mm Hg) and T = absolute temperature in Kelvin. Ki includes a volumetric flow density correction for the free float Rotameter flowmeter used.

Breath Gas Measurement

Endexpired breath gas was collected using a collapsible tube with an open end.[3] Carbon dioxide production in the calorimeter, measured with an infrared analyzer, was used as an estimate of individual alveolar ventilation rates. Assuming the CO_2 concentration in alveolar air is physiologically kept close to 40 mm Hg, and endexpired air represents the gas concentrations in the alveolar space, the following formula was used to calculate breath excretion rates for H_2 and CH_4:

$$RB = ([H_2/CH_4]b - [H_2/CH_4]c) \times RCO_2 \times P/40$$

where RB = breath excretion rate, [ ] = gas concentration, b = breath, c = calorimeter, and RCO_2 = CO_2 production rate.

Hydrogen Analysis

A GMI Exhaled Hydrogen monitor (GMI Medical LTD, Renfrew, Scotland) was modified to read to 0.1 parts per million (ppm), and results were displayed on an external chart recorder to increase resolution of the readings. All measurements were paired with a corresponding fresh air sample. Calibration was with 52.0 ppm standard gas, linearity was found between 0 and 200 ppm, measured using dilutions of pure H_2.

Methane Analysis

Samples were collected as described for H_2 and measured using a PYE 104 gaschromatograph (flame ionisation detector, Poropak Q packed column; PYE Instruments, Cambridge, England). For calibration, standard gas mixtures of 5.2 ± 0.5 ppm and 48 ± 2.4 ppm CH_4 were used. Linearity was found between 1.7 ppm (room air concentration) and 48 ppm. Samples with higher CH_4 concentrations were diluted with nitrogen before measurement.

Calorimeter gas concentrations ranged from 0.0 to 18.2 ppm for H_2 and from 1.7 to 11.0 ppm for CH_4. The coefficient of variations of duplicate measurements was 8.2% for

H_2 and 7.7% for CH_4. Recovery experiments were performed simulating both continuous (from breath) and pulsed (from flatus) production. Single doses of 50 mL and 100 mL of both pure H_2 and CH_4 were injected into the calorimeter. Recovery was 98% and 103% for 50 mL and 100 mL of H_2, respectively, and 100% and 106% for 50 mL and 100 mL of CH_4, respectively. In a separate experiment using a calibrated pump, pure hydrogen was continuously infused at rates of 0.26 mL/min and 1.04 mL/min into the calorimeter for 8 hours each to reach equilibrium (98%) between ingoing and outgoing gas. The volumes of H_2 recovered were 96% and 100% during the lower and the higher flow rates, respectively.

Hydrogen Equivalents

Methanogenic bacteria from the human colon consume 4 mol of hydrogen to form 1 mol of methane: $4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$.[21] An H_2 equivalent (H_2E) was therefore calculated for methanogenic subjects as H_2E (mL) = $4 \times CH_4$ production (mL) + H_2 production (mL), thus allowing both the H_2 used in methanogenesis and the H_2 excreted to be summed.

Starch Analysis

Starch in bananas and in feces was measured as the increase in glucose observed when samples were incubated with amyloglucosidase after gelatinization and incubation with α-amylase and pullulanase as previously described.[10]

Bacterial Counts and Microbial Activity

Fecal slurries were prepared immediately after collection by diluting the samples in anaerobic sodium phosphate buffer (0.1 mol/L; pH 7.0) to a final concentration of 5% (wt/vol). Substrates (glucose, lactose, sucrose, lactulose, mucin, starch, pectin) were added to 60 mL of slurry in a 75 mL serum bottle to give a final concentration of 0.2% (wt/vol). The bottles were gassed out with argon, and then incubated at 37°C on an orbital shaker. H_2, CH_4, and CO_2 concentrations were measured by gas chromatography as described by Allison and McFarlane,[22] and hydrogen sulfide production in the slurry was determined using a colorimetric method after precipitation of S^{2-} in 10% (wt/vol) zinc acetate solution.[23] Viable counts of sulfate-reducing bacteria were enumerated using the agar shake dilution method of Pfennig et al.[24] with media and conditions of cultivation described by Gibson et al.[16] Viable counts of methanogenic bacteria were enumerated using the roll tube technique of Miller and Wolin.[25] For determination of sulfate reduction rates the $^{35}SO_4$ core injection and distillation method of Jørgensen[26] was used. Rates of acetogenesis were measured using $H^{14}CO_3$ as described by Jones and Simon.[27]

Statistics

Variations are expressed as standard error of the mean. The *t* test for independent samples was used for unpaired samples and the Wilcoxon test for paired samples.

Results

Hydrogen Excretion

Basal rates in all subjects were constantly low at 35.0 ± 6.1 mL/day (Figure 1). All subjects except the one with the highest CH_4 excretion showed a marked response to all lactulose doses. Excretion started within 60 minutes, and basal rates were reached again at 15, 17, and 18 hours after the administration of 7.5 g, 15 g, and 22.5 g lactulose (Figure 1). The volumes of total gas excreted after the lactulose doses are shown in Table 1. When expressed as the increment over basal volumes, H_2 from 7.5 g, 15 g, and 22.5 g of lactulose was 88 ± 22 mL, 227 ± 60 mL, and 321 ± 79 mL (Figure 2). H_2 excretion for all subjects and doses was 13.5 ± 2.2 mL/g lactulose (range, 0.5–43 mL/g), with a mean CV of 32%. In reproducibility studies in 4 subjects given 15 g of lactulose on two occasions, total daily H_2 was 873/641 mL, 274/198 mL, 228/242 mL, 51/63 mL (CV 8.6%).

Hydrogen From Polysaccharides

The amount of starch in the bananas given was 28.6 ± 3.7 g. Starch recovered in the 5-day fecal collections after the banana meal was 0.65 ± 0.3 g. It has been shown in ileostomists that about 80% of

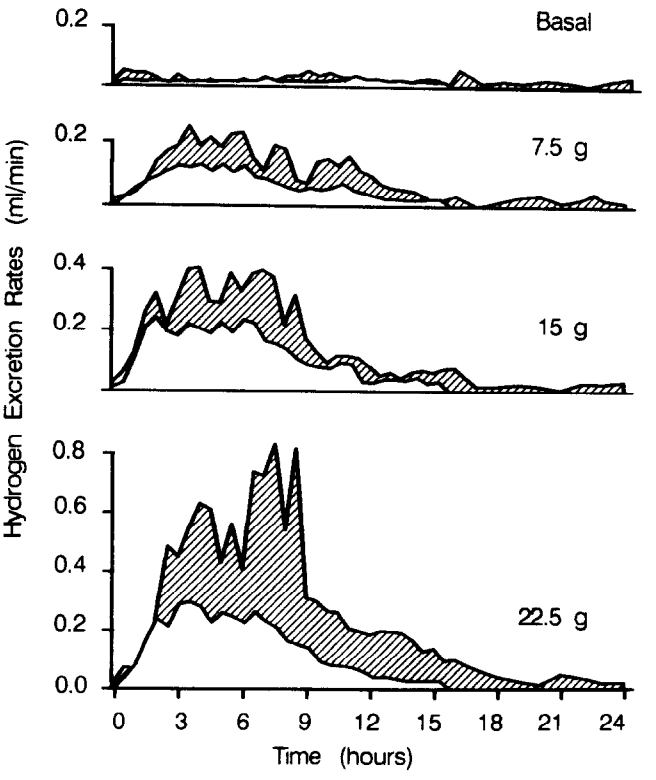

Figure 1. Breath and total H_2 excretion rates after lactulose, 0 g (basal), 7.5 g, 15 g, and 22.5 g. 0 hours = 8 AM (when the test meal was fed; n = 10).

Table 1. Total H_2 and Methane Production From Lactulose

	n	Basal (mL/24 h ± SEM)	7.5 g (mL/24 h ± SEM)	15 g (mL/24 h ± SEM)	22.5 g (mL/24 h ± SEM)
H_2 all subjects	10	35 ± 6 (5 – 70)	123 ± 20 (54 – 279)	262 ± 63 (57 – 757)	356 ± 82 (81 – 967)
H_2 nonmethanogens	6	30 ± 4 (5 – 70)	148 ± 27 (75 – 279)	327 ± 88 (69 – 757)	427 ± 116 (119 – 967)
H_2 methanogens	4	41 ± 6 (19 – 48)	85 ± 11 (54 – 116)	166 ± 33 (57 – 228)	250 ± 29 (81 – 389)
CH_4 methanogens	4	149 ± 37 (37 – 218)	200 ± 47 (92 – 280)	246 ± 68 (57 – 372)	306 ± 84.8 (152 – 527)
H_2 equivalent methanogens	4	637 ± 131 (273 – 905)	886 ± 160 (448 – 1210)	1150 ± 212 (440 – 1540)	1476 ± 261 (882 – 2190)

NOTE. Data expressed as mL/24 h ± SEM (range).

banana starch escapes small intestinal assimilation.[18] Thus, it was calculated that 22.9 g of the banana starch fed in this study was delivered to the colon, 22.2 g of which was fermented. H_2 excretion rates are given in Table 2. Overall amounts were much lower than with lactulose at 4.4 mL/g starch (range, 0.5–7.8). To compare the H_2 from the starch with that from the lactulose, excretion was expressed as a percentage of the H_2 excreted per gram of lactulose for each individual. Banana starch yielded 38.8% ± 9.2% of the volume excreted from an equivalent dose of lactulose (Figure 3). H_2 excretion from pectin is also shown in Table 2. Overall rates were even lower than from starch. Pectin yielded 14.1% ± 3.2% of the H_2 from an equivalent dose of lactulose (Figure 3). Breath H_2 concentration after a test meal of 212 g white bread (100 g starch) rose to a maximum of 19 (10–32) ppm after 8 hours,

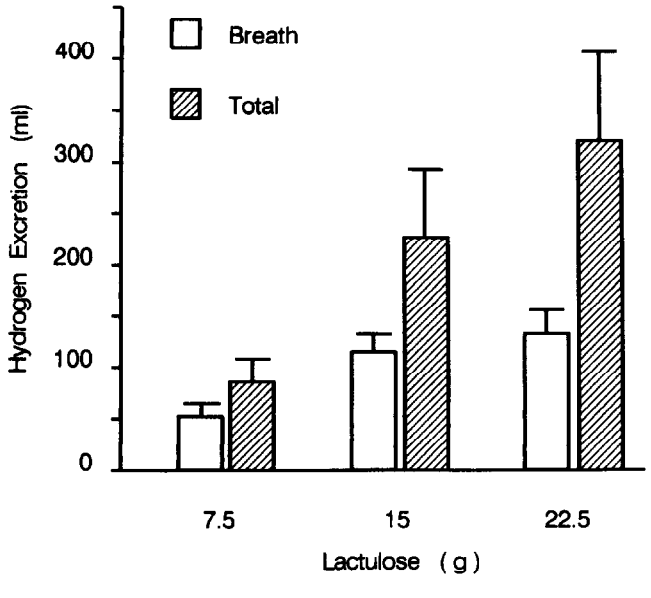

Figure 2. Total (▨) and breath (□) excretion of H_2 from lactulose (increments after subtraction of basal excretion; n = 10).

but average breath H_2 was low and the volumes exhaled were equivalent to those exhaled with 2.5 g of lactulose by the same subjects.

Methane Excretion

Basal CH_4 in 4 subjects was 144 ± 43 mL/day. After 7.5 g, 15 g, and 22.5 g lactulose, CH_4 excretion was 51.3 ± 5.5 mL, 97.3 ± 18.4 mL, and 157.5 ± 36.3 mL above baseline (Figure 4). The hydrogen equivalent calculated for the methanogenic subjects was 34.9 ± 4.6 mL/g lactulose while hydrogen excretion was only 7.9 ± 1.5 mL/g ($P < 0.01$). The H_2 excreted in the nonmethanogenic group, 17.3 ± 3.2 mL/g, was significantly less than the hydrogen equivalent in methane producers ($P < 0.02$).

Breath Gas Versus Total Excretion

To assess how much of the total hydrogen excreted is exhaled in breath, volumes exhaled during the whole measuring period after lactulose were correlated with the corresponding total excretions. Figure 5 shows that breath H_2 excretion was not a constant proportion of total excretion. About 65% of total H_2 was expired in breath at excretion rates up to 200 mL/day. Over this, the proportion decreased asymptotically to plateau at daily volumes of more than 500 mL/day. The overall mean proportion of daily H_2 excretion after lactulose excreted expired in breath was 58% with an individual range from 23%–97%. Methane was released in a similar pattern but because maximal volumes produced were less than observed with hydrogen, breath excretion did not reach a plateau (Figure 5).

Bacterial Counts and Microbial Activity

Hydrogen concentrations in fecal slurries increased in all cultures to a maximum after 8 hours of incubation. H_2 accumulation varied significantly among the different substrates (Figure 6) with pectin

Table 2. Hydrogen Production From Starch and Pectin in Relation to Production After a Comparable Lactulose Dose

Subject no.[a]	Starch fermented (g)	Pectin fermented (g)	H_2 from starch (mL/g)	H_2 from pectin (mL/g)	H_2 from lactulose (mL/g)[b]	Starch % of lactulose	Pectin % of lactulose
1	26.5	20	0.5	4.6	1.3	38.5	18.0
2	24.8	—[a]	8.0	—[a]	10.7	74.8	—[a]
3	19.7	20	6.2	0.6	15.6	39.7	3.5
4	11.7	20	5.5	4.4	20.2	25.8	22.0
5	—[a]	20	—[a]	8.4	43.1	—[a]	19.5
6	30.7	20	1.8	0.8	11.1	14.3	7.6

[a]Subjects B and E were given only starch or pectin, all other subjects had both.
[b]Hydrogen from lactulose was calculated as the average volume released per gram from all doses.

and starch showing only 12% and 27% of the rate measured with lactulose. H_2 concentrations then decreased steadily until the end of the incubation period. This pattern was the same for both CH_4 producers and nonmethanogenic subjects. All other parameters examined showed distinct differences between these two groups (Table 3). CH_4 gas from the fecal slurry was found only in the four methane producers. All of these individuals carried viable populations of methanogenic bacteria (MB), while the nonmethanogenic subjects showed no CH_4 in slurry gas and no detectable MB. Sulfate reducing activity and sulfide production were substantially lower in methanogenic subjects. Viable counts of sulfate reducing bacteria (SRB) were found in all nonmethanogenic subjects, but only one of the methane producers had SRB in his feces and this was on one single occasion only when low numbers were found. Rates of acetogenesis were low in all subjects, and there were no significant differences between methanogenic and nonmethanogenic subjects (Table 3).

Discussion

This study is the first to measure directly both total and breath excretion of hydrogen and methane in man over an extended period of time under near physiological conditions. There are no data directly comparable with the present study, and in only a few papers is total gas excretion reported and compared with breath excretion. Calloway et al.[5,28,29] measured H_2 in breath and in flatus using rectal intubation. They found 50%–100% of total H_2 excretion exhaled in breath. Levitt et al.[30] perfused the bowel with inert gas and estimated that as little as 14% of total H_2 excretion was in breath, this figure being fairly uniform for all subjects and production rates. However, the present study shows that the proportion of H_2 excreted in breath is not constant but depends on total excretion rates. Overall, 58% of the H_2 gas is excreted in breath, with 65% at low production rates (<200 mL/day) and 25% at high rates (>500 mL/day) (Figure 5). Levitt has shown that transport of gases

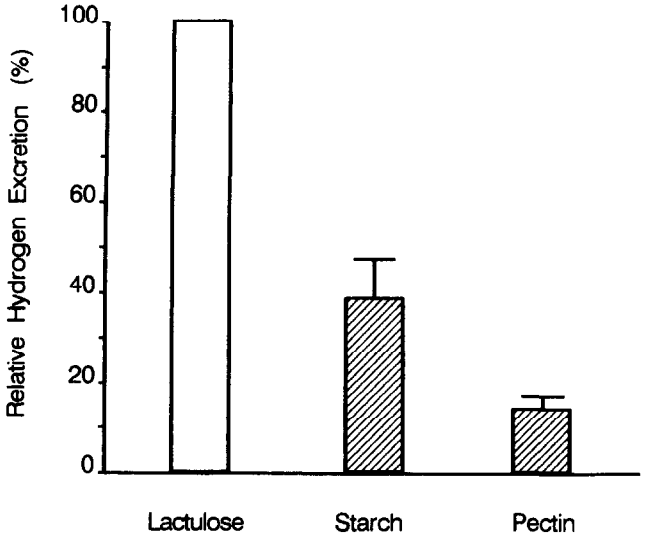

Figure 3. H_2 from banana starch and pectin. Total excretion per 24 hours compared with the hydrogen produced from the equivalent lactulose dose by the same subjects.

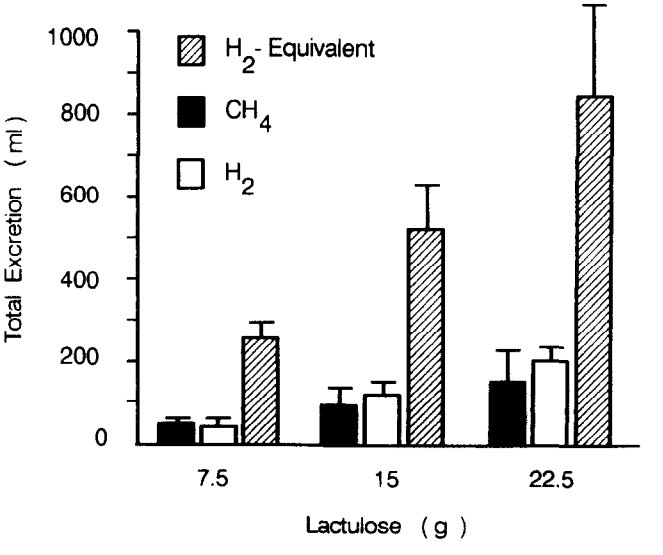

Figure 4. Total hydrogen, methane, and hydrogen equivalent from lactulose in methanogenic subjects (n = 4).

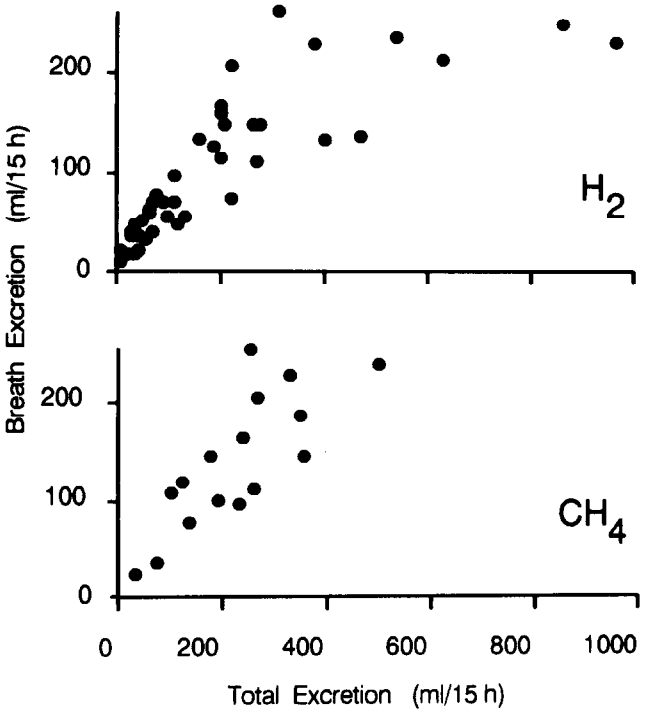

Figure 5. Breath versus total excretion of H_2 and CH_4 from lactulose (all subjects and lactulose doses).

from the intestinal lumen is limited by mucosal diffusion and, because the poor solubility of hydrogen in water, by intestinal blood flow.[31] Gas produced rapidly will therefore accumulate. Large volumes of free gas are not well tolerated in the gut and are expelled as flatus, thus reducing the proportion excreted in breath. Small volumes are more likely to remain in the lumen and diffuse almost completely into the bloodstream. Thus if a relatively slowly fermented substrate, such as starch or fiber, is compared with a rapidly fermentable carbohydrate such as lactulose, its breakdown will be overestimated because a greater proportion of total H_2 will be excreted in breath. CH_4 showed similar excretion kinetics to H_2.

Lactulose is not assimilated in the small intestine of man but is completely broken down in the colon.[32] It is therefore used as a reference substance in breath studies to quantify carbohydrate malabsorption.[8,33–35] However, as a disaccharide it is representative of only a minor fraction of the carbohydrate reaching the colon on an average diet, which is mostly polysaccharide, either starch or nonstarch polysaccharides (NSP; dietary fiber).[1,36,37] Therefore, it is perhaps not surprising that estimates of wheat starch malabsorption using the lactulose standardized H_2 breath test range from 7%–20%,[7,8,38] whereas in the present study using breath H_2 measurements it was only 2.5%. Probably very little wheat starch reaches the colon.[10] Therefore, to test the excretion of H_2 from starch in the present study, banana starch was used. Starch from raw bananas is present in granules almost totally resistant to pancreatic amylase, and more than 80% escape small intestinal assimilation.[18] Although the amount predicted to have reached the colon was 22.9 g, H_2 production was low, equivalent to only 8.6 g of lactulose, yet fecal collections showed that the starch was almost completely broken down. Similarly, relatively little hydrogen was excreted from pectin (Figure 3). Pectin was chosen as a typical constituent of dietary fiber. It is known to be undigested in the small intestine, and we have previously shown that it is extensively fermented and completely degraded in the human colon.[3,39] However, the 20 g given yielded only 14% of the H_2 excreted after a comparable dose of lactulose. Correspondingly, H_2 production rates in fecal slurry were substantially lower for the polysaccharides than for sugars (Figure 6).

These results show that different carbohydrates do not release a constant amount of H_2 per gram fermented either in vivo or in vitro. There are a number of possible reasons for this. As each carbohydrate requires a variety of bacterial enzymes for its breakdown,[40] a distinct consortium of bacteria will be best capable of fermenting it. The metabolic pathways preferred by these bacteria lead to different patterns of products.[40] In vitro fermentation studies have shown that various polysaccharides yield different proportions of the main end products, short chain fatty acids. During fermentation to more oxidised products, such as acetate, H_2 is generated as an electron acceptor while in the formation of more reduced products such as propionate net hydrogen consumption occurs.[41]

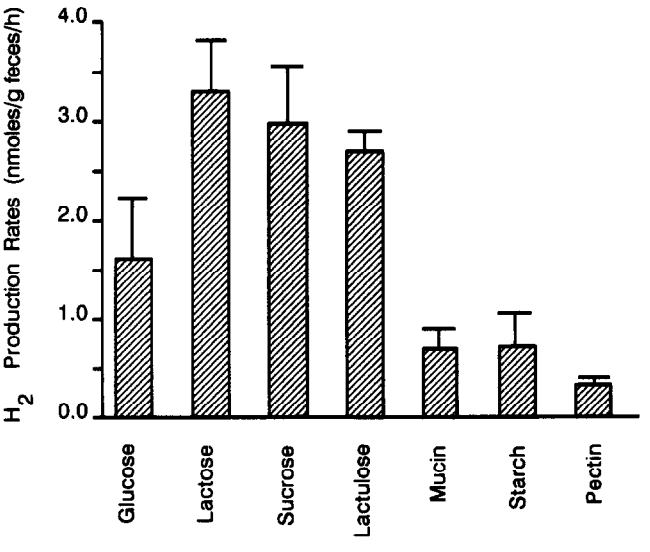

Figure 6. Hydrogen production rates in fecal slurries with various carbohydrates as fermentable substrates.

Table 3. Metabolic Activity and Viable Counts of Bacteria in Feces of Methanogenic and Nonmethanogenic Subjects

	Sulfate reduction rate ($nmol \cdot h^{-1} \cdot g^{-1}$)[a]	Sulfide production rate ($\mu mol \cdot h^{-1} \cdot g^{-1}$)[a]	Acetate production rate ($nmol \cdot h^{-1} \cdot g^{-1}$)[a]	Methane production rate ($\mu mol \cdot h^{-1} \cdot g^{-1}$)[a]	Viable counts (log 10/g)[a]	
					SRB[b]	MB
Methanogenic subjects	7.9 ± 2.0 (2.9 – 16.5)	0.28 ± 0.04 (0.22 – 0.40)	2.1 ± 0.81 (0.1 – 3.3)	0.92 ± 0.35 (0.24 – 2.06)	0 —	8.3 ± 0.3 (7.8 – 9.5)
Nonmethanogenic subjects	58.7 ± 5.6 (43.2 – 81.2)	1.41 ± 0.11 (1.2 – 1.8)	3.2 ± 0.83 (1.6 – 4.7)	0	7.2 ± 0.3 (5.5 – 8.2)	0

[a]Wet weight of feces.
[b]One methanogenic subject on one occasion had log 3.3 SRB in his feces.

The amount of hydrogen excreted also depends on whether H_2 is consumed in other reactions in the colon. This study provides clear evidence for such mechanisms. Although theoretical fermentation equations suggest an H_2 production of 106 mL per gram of carbohydrate degraded,[42] as little as 13.5 mL was found in this study per gram of lactulose and only 4.4 mL and 3.8 mL per gram of starch and pectin, respectively. Molecular H_2 is produced by anaerobic bacteria to dispose of reducing equivalents. However, high partial pressures of H_2 inhibit the reoxidation of reduced $NADH_2$, a coenzyme essential for bacterial hexose breakdown.[21] Therefore, anaerobic ecosystems such as the rumen and aquatic sediments always involve the activities of H_2-consuming organisms.

In the ruminant, the major pathway for the H_2 disposal is reduction of CO_2 to CH_4.[21] Similarly, in the human colon, methanogenic bacteria may use CO_2 and H_2 to form CH_4.[43] The present study clearly shows an increase in both breath and total CH_4 excretion after lactulose ingestion in methanogenic subjects (Figure 4). When total H_2 equivalents were calculated, it was found that methanogenic individuals converted three times as much H_2 to CH_4 as they excreted as H_2 (Table 1). Thus, methanogenesis is a powerful H_2-consuming process in the colon, capable of reducing hydrogen excretion by 75%.

However, about half of a European population does not show significant CH_4 production, and 6 of the 10 subjects in this study were nonmethanogenic. Hydrogen excretion in this group was only half of the hydrogen equivalent in methanogenic subjects. Assuming a similar amount of H_2 is produced in methanogenic and nonmethanogenic subjects, this indicates a considerable volume of H_2 is catabolized even in the absence of methanogenesis. Sulfate is known to be an important terminal electron acceptor in anaerobic environments.[44,45] Recently, the presence of sulfate reducing bacteria in human feces has been shown and correlated with an absence of methanogenesis.[16] Competition between these two types of bacteria occurs in vitro.[46] In the present study, high sulfate-reducing activity was shown in all nonmethanogenic subjects while only one of the methane producers showed low levels of sulfate reduction on one occasion at a time when his methane production was low. Accordingly, viable counts of sulfate reducing bacteria were detected only in the feces of nonmethanogenic subjects, and only methane producers carried methanogenic bacteria (Table 3). Further evidence for uptake of H_2 other than by methanogenesis is seen in the pattern of basal gas production on the polysaccharide free diet. In methanogenic individuals, there was steady methane production presumably from metabolism of endogenous substrates such as mucus,[47] but there was almost no gas production in nonmethanogenic subjects. In this group, the corresponding reducing equivalents were probably disposed of by the reduction of endogenous sulfate because in vitro experiments have shown that bacterial sulphate reduction is stimulated by sulfated mucopolysaccharides.[48,49] These findings indicate that sulfate reduction is an important pathway for terminal electron disposal in the colonic fermentation and an alternative to methanogenesis.

A third potential mechanism for H_2 uptake in fermentation is acetogenesis by reduction of CO_2 to acetate.[17] There was little acetogenic activity in the feces of our subjects (Table 3), and no differences were found between the methanogenic and the sulfate-reducing group. The pH optimum for acetogenesis is about 6 and at the approximately neutral pH of feces, acetogenic bacteria will be outcompeted by both methanogenesis and sulfate reduction.[50] Thus, colonic acetogenesis is probably only significant in the cecum at a more acid pH, and our methods were therefore not suitable for its detection.

Conclusions

The proportion of H_2 excreted in breath was variable and depended on production rates. Fermentation of lactulose generated more H_2 than did resistant starch or pectin. Methanogenic or sulfate reducing bacteria consume substantial volumes of

H_2. Thus, the composition of the colonic flora determines, in part, the nature and amount of gas produced in the bowel. For these reasons the lactulose standardized hydrogen breath test is not suitable for accurate quantification of malabsorbed polysaccharides.

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