APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1999, p. 2807–2812 Vol. 65, No. 7
0099-2240/99/$04.00+0
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Changes of Fermentation Pathways of Fecal Microbial Communities Associated with a Drug Treatment That Increases Dietary Starch in the Human Colon

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Received 8 December 1998/Accepted 10 March 1999

Acarbose inhibits starch digestion in the human small intestine. This increases the amount of starch available for microbial fermentation to acetate, propionate, and butyrate in the colon. Relatively large amounts of butyrate are produced from starch by colonic microbes. Colonic epithelial cells use butyrate as an energy source, and butyrate causes the differentiation of colon cancer cells. In this study we investigated whether colonic fermentation pathways changed during treatment with acarbose. We examined fermentations by fecal suspensions obtained from subjects who participated in an acarbose-placebo crossover trial. After incubation with [1-^{13}C]glucose and $^{12}CO_2$ or with unlabeled glucose and $^{13}CO_2$, the distribution of ^{13}C in product C atoms was determined by nuclear magnetic resonance spectrometry and gas chromatography-mass spectrometry. Regardless of the treatment, acetate, propionate, and butyrate were produced from pyruvate formed by the Embden-Meyerhof-Parnas pathway. Considerable amounts of acetate were also formed by the reduction of CO_2. Butyrate formation from glucose increased and propionate formation decreased with acarbose treatment. Concomitantly, the amounts of CO_2 reduced to acetate were 30% of the total acetate in untreated subjects and 17% of the total acetate in the treated subjects. The acetate, propionate, and butyrate concentrations were 57, 20, and 23% of the total final concentrations, respectively, for the untreated subjects and 57, 13, and 30% of the total final concentrations, respectively, for the treated subjects.

Acarbose, an oligosaccharide formed by strains of the genus *Actinoplanes* (23), is an α-glucosidase inhibitor that is used to treat non-insulin-dependent diabetes mellitus (2). This compound inhibits starch digestion in the small intestine (2, 5, 6). The increased amount of colonic starch selects for growth of starch-using bacteria (22). The ratio of viable starch-hydrolyzing bacteria to total viable anaerobic bacteria in feces increases (22), and fecal suspensions produce more butyrate from starch (22). Starch fermentation by colonic bacteria produces large amounts of butyrate (3, 4, 10, 21). Enhancement of colonic starch fermentation by acarbose treatment increases the butyrate concentration and the proportion of butyrate in the short-chain fatty acid (SCFA) component of feces (18, 22). Butyrate is an energy source for colonic epithelial cells (17), and this compound promotes differentiation and inhibits growth of colon cancer cells (1, 19).

Analysis of the labeling of fermentation products obtained from isotopically labeled substrates can reveal the metabolic pathways used by colonic microbes to form products. Using radioactive glucose and CO_2 as substrates, Miller and Wolin showed previously that the colonic flora of two adults used the Embden-Meyerhof-Parnas (EMP) pathway as the primary metabolic route for SCFA production (15). The flora also formed considerable amounts of acetate by reducing CO_2 rather than by direct formation from glucose (15). Wolin et al. used ^{13}C-labeled glucose and CO_2 to study fermentations by the fecal flora of breast-fed infants (25, 26). Although the products differed from the products found in adults, the flora of two breast-fed infants less than 1 month old used the EMP pathway to metabolize glucose. After 5 months, reexamination of one infant showed that a totally different fermentation pathway, used only by bifidobacteria, had replaced the EMP pathway. In contrast to the adult flora, the flora of the infants was incapable of reducing CO_2 to acetate.

We examined these pathways in a larger group of adults to investigate possible changes caused by acarbose treatment. The isotopic composition of products of fermentations by the fecal bacteria of subjects who participated in an acarbose-placebo crossover trial with a 3- to 4-month rest period between treatments was determined. We incubated fecal suspensions with [1-^{13}C]glucose and $^{12}CO_2$ or with unlabeled glucose and $^{13}CO_2$ and examined the distribution of ^{13}C in the product C atoms. The data showed that acarbose treatment resulted in large decreases in the reduction of CO_2 to acetate, as well as the formation of propionate from glucose. Butyrate formation from glucose increased considerably with acarbose treatment. The EMP pathway was the major pathway used for fermentation with or without acarbose treatment.

MATERIALS AND METHODS

Subjects and fecal suspensions. Fermentations were examined by using a fecal suspension from each of 40 patients who participated in an acarbose-placebo crossover trial (unpublished data). Baseline samples were taken from 21 subjects before they were given either acarbose (100-mg tablet daily) or a placebo (100-mg tablet daily). Samples were then taken from 10 subjects after they received acarbose for 4 months and from 6 subjects who received the placebo for 4 months. A 3- to 4-month rest period followed the 4 months of acarbose or placebo regimen before a crossover of each subject's treatment was begun. We examined baseline samples from three subjects after the rest period. Statistical

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analysis showed that the data obtained during the baseline and placebo regimens were not different from each other. The results obtained from the baseline and placebo samples were combined into one data set which represented subjects that did not receive acarbose (designated the Aneg group). These results were compared with results obtained with samples from acarbose-treated subjects (designated the Apos group).

Suspensions of feces were prepared in anaerobic dilution solutions under CO_2 as described previously (14, 20). The suspensions were kept at 4°C and were used within 24 h of collection. Anaerobic conditions were maintained by using the serum bottle modification of the Hungate technique (13). Duplicate (5-ml) portions were dried to constant weight to determine the fecal dry matter content. The human fecal fermentation protocols were reviewed and approved by the New York State Department of Health Institutional Review Board. The protocols used for the acarbose-placebo crossover trial were reviewed and approved by the Mary Imogene Bassett Hospital Institutional Review Board.

Fermentations. Fermentations of glucose were performed in anaerobic culture tubes (18 by 150 mm; Bellco Glass Inc., Vineland, N.J.). Glucose (50 mg) was added as a dry powder prior to insertion of butyl rubber stoppers and sealing with aluminum seals. The tubes were gassed with 80% N_2–20% CO_2. After 5-ml portions of 76 mM $NaHCO_3$ from a serum bottle with a 100% N_2 atmosphere were injected, the tubes were cooled to 0°C in ice water. Formate was added to some fermentations by adding 0.2 ml of a 0.5 M sodium formate solution from a serum bottle with a 100% N_2 atmosphere. Fermentation was started by injecting 5.0 ml of a suspension. After incubation with rotation for 24 h at 37°C, the tubes were boiled for 10 min. The contents were either analyzed immediately or frozen at −20°C and thawed before gas samples were removed for gas chromatography and mass spectrometric analyses. Suspensions were then acidified by adding 0.5 ml of 5 N H_2SO_4 and were centrifuged at 16,000 × *g* for 15 min. Supernatants were analyzed to determine their fermentation product and residual glucose contents.

Fermentation analyses. Soluble fermentation product and glucose contents were determined by high-performance liquid chromatography procedures (9) as described by Wolin et al. (26). H_2 and CH_4 were quantified by using previously described gas chromatographic procedures (15). Mass spectral analyses to determine $^{13}CO_2$ contents were carried out with a model 5890A gas chromatograph (Hewlett-Packard, Palo Alto, Calif.) equipped with a model 5970 Series mass selective detector (Hewlett-Packard) as previously described by Wolin et al. (26).

NMR. Nuclear magnetic resonance (NMR) spectra were acquired with a model XL-300 spectrometer (Varian Associates, Walnut Creek, Calif.) operating at 75.43 MHz. Pulses of 36° were used, and the delay time was 1 s. The number of transients ranged from 5,000 to 40,000. The NMR locking material was deuterium oxide. All spectra were recorded at 25°C with a spectral width of 16,000 Hz.

Chemical shifts for SCFA were assigned directly by using previously published values and samples of labeled acetate. To measure the percentage of enrichment of ^{13}C, we used natural abundance dioxane (reagent grade and distilled from lithium aluminum hydride; purity, >99.9%) as a standard. Dioxane gives a strong singlet at 67.4 ppm that is separate from the signals of the species studied under the conditions used for the NMR analyses. Pure unlabeled dioxane (5 μl) was sealed in a capillary tube with D_2O, and this capillary tube was inserted into the NMR sample tubes as a reference each time that a spectrum was acquired. We prepared three standard samples of labeled acetate with the same concentration, 53.63 mM. Two of these samples were singly labeled with ^{13}C at either C-1 or C-2; the third was doubly labeled. All three samples had an enrichment of 31.01%. Their spectra were acquired under identical conditions with the reference capillary tube mentioned above in the NMR tube. All three samples exhibited agreement in the ratio of peak intensity of C-1 or C-2 to dioxane intensity, which was determined to be 2.1 or 3.1 (designated R_o). The spectrum of a fermentation sample was acquired with the same capillary tube, and the ratio of its peak intensity to dioxane intensity (R) was obtained. To convert this ratio to the millimolar concentration of ^{13}C carbon atom, C, the following equation was used: $C = R/R_0 \times 0.3101 \times 53.63$. Concentrations of ^{13}C-labeled methyl, ^{13}C-labeled methylene, and ^{13}C-labeled carboxyl of propionate and butyrate were calculated similarly by using $R_o = 2.1$ for the methyl and methylene groups and $R_o = 3.1$ for the carboxyl groups. This method was verified by determining the concentrations ^{13}C in the carbons of propionate and butyrate with known concentrations of ^{13}C in the respective C atoms.

NMR analysis of fermentation samples. Acidified fermentation supernatant was added to the NMR tube without any solvent. The same dioxane reference capillary tube was also put into the tube each time. The products were identified and the enrichment of ^{13}C was determined by the methods described above.

Materials. ^{13}C-labeled C-1, C-2, and doubly labeled sodium acetate were obtained from MSD Isotopes (Montreal, Canada) and were 99% enriched. ^{13}C-labeled glucose, sodium bicarbonate, and sodium formate (99% enriched) were purchased from Cambridge Isotope Laboratories (Woburn, Mass.). All other chemicals were reagent grade or better.

Data analysis. Unless stated otherwise, the means obtained for the treatments are presented below. Excel (Microsoft Corporation, Redmond, Wash.) was used to perform Student's *t* test in order to determine the significance of differences between means.

TABLE 1. Amounts of SCFA produced in 24 h from [1-^{13}C]glucose and endogenous substrates

Acid	Subjects[a]			
	Aneg group		Apos group	
	Concn (mM)	%	Concn (mM)	%
Acetate	42.4 (7.8)[b]	56.8	46.1 (9.1)	56.9
Propionate	14.9 (5.9)	20.0[c]	10.8 (5.2)	13.3[c]
Butyrate	17.4 (5.9)[d]	23.2[e]	24.1 (10.3)[d]	29.8[e]

[a] The values are the averages of the values from two or three fermentations for each sample. The percentages are percentages of the total concentration of the three SCFA.
[b] The values in parentheses are standard deviations.
[c] The difference between the Aneg group value and the Apos group value is significant ($P < 0.002$), as determined by Student's *t* test.
[d] The difference between the Aneg group value and the Apos group value is significant ($P < 0.02$), as determined by Student's *t* test.
[e] The difference between the Aneg group value and the Apos group value is significant ($P < 0.02$), as determined by Student's *t* test.

RESULTS

Fermentation of glucose and endogenous substrates. Table 1 shows the SCFA formed from glucose and endogenous substrates in the fecal suspensions and the mean concentrations obtained for the Aneg and Apos groups. The sum of the C atoms in the products was 19.4% greater than the number of C atoms in the added glucose for the Aneg group and 32.7% greater for the Apos group. Additional products formed by fermentation of endogenous substrates were the probable sources of the additional C. The starch concentration in feces of healthy volunteers increased from 68.5 to 241.2 μmol per g (dry weight) of feces when the volunteers received 200 mg of acarbose per day (22). Table 1 shows that the amount of butyrate formed was greater in the Apos group and that butyrate accounted for 23% of the SCFA for the Aneg group and 30% of the SCFA for the Apos group. Although the amount of propionate formed was smaller in the Apos group, the difference between the two groups was not statistically significant; however, the difference in the percentages of the SCFA (20% for the Aneg group and 13% for the Apos group) was significant (Table 1).

Incorporation of $^{13}CO_2$. The flora in the fecal suspensions incorporated $^{13}CO_2$ mainly into the methyl and carboxyl C atoms of acetate and the carboxyl C atoms of propionate after incubation with $^{13}CO_2$ and unlabeled glucose (Table 2). The other C atoms of propionate and the C atoms of butyrate contained much smaller amounts of ^{13}C. Incorporation into both C atoms of acetate resulted from the reduction of CO_2 to both the methyl and carboxyl C atoms that is characteristic of the Wood-Ljungdahl pathway of formation of acetate from CO_2 (Fig. 1). More $^{13}CO_2$ was incorporated into the carboxyl group than into the methyl group of acetate (Table 2). Exchange of $^{13}CO_2$ with the unlabeled carboxyl of acetate formed from glucose or endogenous substrates could explain the differential labeling of the two C atoms. Acetyl S-coenzyme A (acetyl-SCoA) synthase catalyzes the exchange of CO_2 with the carboxyl group of acetate (16).

Incorporation of $^{13}CO_2$ into butyrate resulted from the conversion of ^{13}C-labeled acetate to acetyl-SCoA. Interconversion of acetate and acetyl-SCoA is a common process in bacteria and is facilitated by the enzymes acetate kinase (acetate + ATP → acetyl-phosphate + ADP) and phosphotransacetylase (acetyl-phosphate → acetyl-SCoA + inorganic phosphate). Butyrate is usually formed from acetyl-SCoA units produced

TABLE 2. Enrichment of SCFA and CO_2 after incubation with $^{13}CO_2$ and unlabeled glucose

Incorporation of $^{13}CO_2$		Aneg group		Apos group		P value[b]
SCFA	Position	% ^{13}C enrichment of C atom	^{13}C concn (mM)[a]	% ^{13}C enrichment of C atom	^{13}C concn (mM)[a]	
Acetate	CH_3	3.60	1.51[c]	2.08	0.93[d]	<0.0004
	COOH	5.79	2.38[c]	3.02	1.32[d]	<0.00002
Butyrate	CH_3	1.92	0.33	1.10	0.27	
	CH_2	2.48	0.43	1.44	0.36	
	CH_2	1.90	0.32	1.09	0.27	
	COOH	2.79	0.47	1.60	0.40	
Propionate	CH_3	1.19	0.17	0.63	0.08	<0.0002
	CH_2	0.94	0.14	0.46	0.05	<0.0007
	COOH	17.98	2.58	13.86	1.42	<0.002
	CO_2	21.70		26.20		<0.00002

[a] Calculated by multiplying the SCFA concentration shown in Table 1 by the percent ^{13}C enrichment divided by 100.
[b] Probability that the Aneg group and Apos group values are different, as determined by Student's t test.
[c] The methyl and carboxyl group values are significantly different ($P < 9 \times 10^{-13}$), as determined by Student's t test.
[d] The methyl and carboxyl group values are significantly different ($P < 0.0002$), as determined by Student's t test.

from the oxidative decarboxylation of pyruvate (Fig. 2). Exchange occurs between the acetyl-SCoA units formed from labeled acetate produced from $^{13}CO_2$ and the acetyl-SCoA units formed from unlabeled pyruvate. The extents of exchange of ^{13}C-labeled acetyl-SCoA into butyric acid were 16 and 21% for the Aneg and Apos groups, respectively.

Amount of acetyl units formed by CO_2 reduction. The total amount of acetyl units formed from CO_2 was equal to the amount of labeled acetyl units in butyrate and in free acetate after incubation with $^{13}CO_2$. We calculated the amount of acetate and butyrate acetyl units formed from $^{13}CO_2$ from the amount of labeled methyl groups because of possible exchange of $^{13}CO_2$ with the carboxyl carbon of acetate. Incorporation of labeled acetyl units into butyrate was evaluated by determining the ^{13}C content of the butyrate C atoms. The amount of the methyl C of the acetyl units incorporated was determined from the ^{13}C contents of the C-2 and C-4 atoms of butyrate. The ^{13}C concentrations in the different C atoms were almost identical, and the mean was used to calculate the amount of labeled acetyl units in butyrate. This amount was added to the amount in free acetate calculated from the amount of acetate methyl C formed from $^{13}CO_2$.

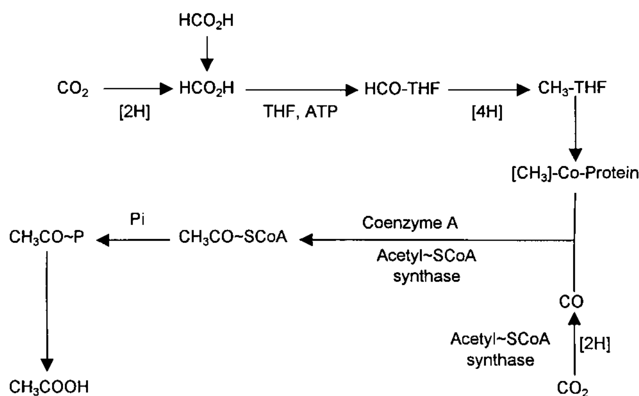

FIG. 1. Wood-Ljungdahl pathway for reduction of CO_2 to acetate. THF, tetrahydrofolic acid; Co-Protein, corrinoid enzyme; Pi, inorganic phosphate (adapted from reference 8 with permission from the publisher).

Since $^{13}CO_2$ and $^{12}CO_2$ were present, four molecular species of acetate were formed. The enzymes of the pathway can use either $^{12}CO_2$ or $^{13}CO_2$ as a source of the methyl and carboxyl carbons of acetate. Table 3 shows the four species of acetate formed from CO_2 and the formulas used to calculate the proportion of each molecular species from the percentage of ^{13}C enrichment of CO_2. The method used to calculate the total concentration of the four species from the concentration of $^{13}CH_3{}^{12}COOH$ is also shown. Table 4 shows the concentrations of acetate produced by the CO_2 reduction pathway. The amount of acetate formed from CO_2 by the fecal fermentations of the Aneg group was 30% of the total amount of acetate produced. This compares with the much lower amount obtained for the Apos group (17% of the total amount of acetate produced).

Relationship between acetate formation from CO_2 and CH_4 production. CH_4 formation in the colon is a CO_2 reduction process, as is the formation of acetate from CO_2. We compared the two processes by studying 21 samples. Ten of the samples (all baseline samples) produced less than 0.26 μmol of CH_4 per ml of suspension, and 11 samples (10 baseline samples and 1 Apos sample) produced more than 4.17 μmol of CH_4 per ml of suspension incubated with glucose and bicarbonate. H_2 was not detected as a final fermentation product in any of the fecal suspensions. The means (and standard deviations) were 0.04 (0.08) and 8.37 (3.17) μmol of CH_4 per ml of suspension for the low- and high-methane-content samples, respectively. The corresponding means (and standard deviations) for production of acetate from CO_2 were 16.40 (4.50) and 11.17 (2.75) μmol per ml of suspension for the low- and high-methane-content samples, respectively, and these values were significantly different ($P = 0.002$), as determined by Student's t test. The differences between the other fermentation measurements (i.e., SCFA formation or formation of labeled products from [1-^{13}C]glucose or $^{13}CO_2$) were not significant.

Carboxylation and CO_2 exchange and propionate production. Incorporation of $^{13}CO_2$ into the carboxyl C of propionate can result from the carboxylation of pyruvate to oxaloacetate in the succinate pathway shown in Fig. 2. After reduction of oxaloacetate to succinate, decarboxylation yields propionate. One carboxyl of succinate is from the carboxyl of pyruvate, and the other is from CO_2. Decarboxylation produces propionate

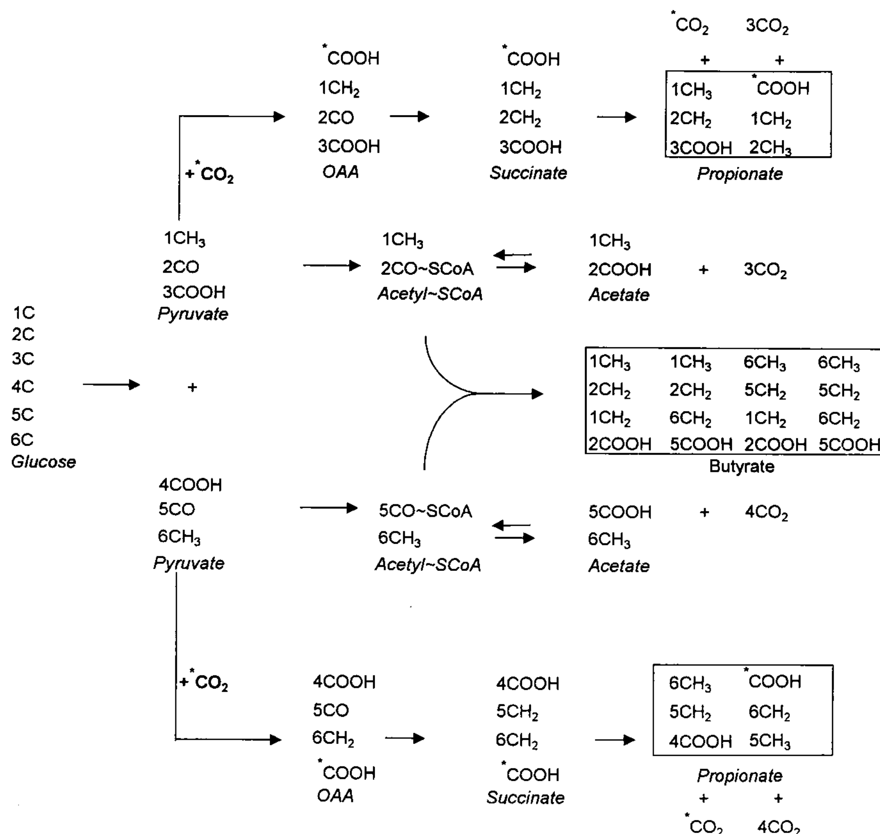

FIG. 2. EMP pathway for glucose decomposition and production of acetate, propionate, and butyrate from pyruvate. The numbers of the C atoms of glucose that are found in the various C atoms of products formed by the pathway are indicated, as are the carbons of propionate that are formed from carbon dioxide (indicated by asterisks). Details of the enzyme reactions between glucose and pyruvate and between pyruvate and acetate, propionate, and butyrate are not shown. For details of enzyme reactions between glucose and pyruvate and between pyruvate and acetate, propionate, and butyrate, see reference 11.

in which one-half of the propionate molecules have a carboxyl C from CO_2 and one-half have a carboxyl C from the carboxyl of pyruvate. CO_2 can also enter the carboxyl C of pyruvate by exchange reactions of enzyme systems that form acetyl-SCoA units and CO_2 from pyruvate (24). Of the 14.95 mM propionate formed by the Aneg group (Table 1), 82% of the carboxyl groups contained ^{13}C, whereas 52% of the 10.81 mM propionate formed by the Apos group (Table 1) contained ^{13}C. Since the level of labeling of the carboxyl by the flora of the Aneg group was significantly greater than 50%, the propionate-forming flora apparently produced substantial exchange of CO_2 into the carboxyl of pyruvate.

TABLE 3. Calculation of the proportions of acetate species formed from CO_2[a]

CO_2 isotopes	Acetate species	% of species
$^{12}CO_2$, $^{12}CO_2$	$^{12}CH_3$ $^{12}COOH$	(% $^{12}CO_2$/100) × (% $^{12}CO_2$/100)
$^{13}CO_2$, $^{13}CO_2$	$^{13}CH_3$ $^{13}COOH$	(% $^{13}CO_2$/100) × (% $^{13}CO_2$/100)
$^{12}CO_2$, $^{13}CO_2$	$^{12}CH_3$ $^{13}COOH$	(% $^{12}CO_2$/100) × (% $^{13}CO_2$/100)
$^{13}CO_2$, $^{12}CO_2$	$^{13}CH_3$ $^{12}COOH$	(% $^{12}CO_2$/100) × (% $^{13}CO_2$/100)

[a] For example, with 75% $^{12}CO_2$ and 25% $^{13}CO_2$, $^{12}CH_3$ $^{12}COOH$ = 0.75 × 0.75 = 0.56 and $^{13}CH_3$ $^{13}COOH$ = 0.25 × 0.25 = 0.06. $^{12}CH_3$ $^{13}COOH$ and $^{13}CH_3$ $^{12}COOH$ each accounted for 0.75 × 0.25 or 0.19 of the total acetate with methyl and carboxyl carbons from CO_2. The total number of micromoles obtained with methyl and carboxyl C from $^{12}CO_2$ $^{13}CO_2$ was calculated from the concentration of the ^{13}C methyl group of acetate and was equal to 1/[(% $^{12}CO_2$/100) × (% $^{13}CO_2$/100) × (mM $^{13}CH_3$)].

Production of SCFA from [1-^{13}C]glucose. Table 5 shows the ^{13}C enrichments of C atoms of the SCFA and CO_2 produced by the fecal fermentations of [1-^{13}C]glucose. The ^{13}C was incorporated primarily into C-2 of acetate, C-2 and C-3 of propionate, and C-2 and C-4 of butyrate. Acetyl-SCoA units produced from [1-^{13}C]glucose by the EMP pathway (Fig. 2) and labeled in the methyl but not the carboxyl C are converted to free acetate. An equivalent amount of unlabeled acetyl-SCoA units and acetate are formed from C-5 and C-6 of glucose. Butyrate formation requires the condensation of two acetyl-SCoA units (Fig. 2). Only one-half of the acetyl groups in butyrate are labeled because of the synthesis of unlabeled acetyl-SCoA units from C-6 and C-5 of [1-^{13}C]glucose. As discussed above, propionate is produced after carboxylation of

TABLE 4. Calculated amounts of acetate formed from the CO_2 reduction pathway in 24 h

Group	Acetate concn (mM) with the following sources of acetate:		% of total from CO_2
	CO_2	CO_2 + substrate C[a]	
Aneg	12.87[b]	42.38	30.36
Apos	7.76[b]	46.10	16.83

[a] See Table 1 for the total amounts of acetate formed by fermentation.
[b] Values are significantly different ($P < 0.002$), as determined by Student's t test.

TABLE 5. Concentrations of ^{13}C in C atoms of SCFA after incubation with [1-^{13}C]glucose

Incorporation of ^{13}C		Aneg group		Apos group		P value[b]
SCFA	Position	% ^{13}C enrichment of C atom	^{13}C concn (mM)[a]	% ^{13}C enrichment of C atom	^{13}C concn (mM)[a]	
Acetate	CH_3	24.09	9.80	21.18	9.59	
	COOH	2.61	1.09	1.40	0.64	<0.0004
Butyrate	CH_3	15.56	2.57	14.07	3.40	<0.0002
	CH_2	0.47	0.11	0.32	0.10	<0.0003
	CH_2	15.00	2.49	13.77	3.34	
	COOH	0.13	0.02	0.00	0.00	<0.04
Propionate	CH_3	13.06	1.87	7.29	0.90	
	CH_2	12.27	1.78	6.78	0.85	<0.03
	COOH	0.00	0.00	0.00	0.00	
	CO_2	2.19		2.44		

[a] Calculated by multiplying the SCFA concentration shown in Table 1 by the percent ^{13}C enrichment divided by 100.
[b] Probability that the Aneg group and Apos group values are different, as determined by Student's *t* test.

pyruvate and formation of succinate (Fig. 2). After decarboxylation of succinate, 50% of the ^{13}C from [1-^{13}C]glucose is in the methyl C and 50% is in the C-2 atom of propionate (Fig. 2). The concentrations of $^{13}CH_3{}^{12}CH_2COOH$ and $^{12}CH_3{}^{13}CH_2COOH$ are the concentrations in the respective ^{13}C-labeled C atoms (Table 5). An equivalent amount of unlabeled propionate is formed from the pyruvate made from the C-6 end of glucose (Fig. 2). The amounts of SCFA calculated from the data in Table 5 were as follows: 20 mM acetate, 7 mM propionate, and 5 mM butyrate for the Aneg group and 19 mM acetate, 4 mM propionate, and 7 mM butyrate for the Apos group. The propionate and butyrate concentrations and the percentages of the three SCFA of the two groups were significantly different ($P < 0.05$), as determined by Student's *t* test.

Production of CO_2 from [1-^{13}C]glucose. The amount of CO_2 formed from the C-1 atom of [1-^{13}C]glucose was calculated from the percentage of $^{13}CO_2$ in the CO_2 found after incubation with [1-^{13}C]glucose or [^{13}C]bicarbonate. After incubation with [^{13}C]bicarbonate, the percentage of the $^{13}CO_2$ was 22.6%, and after incubation with [1-^{13}C]glucose the percentage of $^{13}CO_2$ was 2.3%. The amount of CO_2 formed from the C-1 atom of glucose was 38.8 μmol. The mean percentage of conversion of the C-1 atom of glucose to CO_2 for all of the samples was 10.54% (standard deviation, 0.51%) of the added [1-^{13}C]glucose.

DISCUSSION

NMR analysis of fermentations of samples from 40 subjects provided additional evidence (15) that the Wood-Ljungdahl pathway plays a major role in the production of acetate in the human colon. Bacteria that uses this pathway couple the oxidation of carbohydrates to acetate and CO_2 to the reduction of CO_2 to acetate. They also use other electron sources, such as H_2 produced by other bacteria, to reduce CO_2 to acetate (7). Their contribution to the overall fermentation decreases during acarbose treatment. The acetate formed from CO_2 reduction was 30% of the total acetate formed by the Aneg group and only 17% of the total acetate formed by the Apos group. The contribution of bacteria that produce propionate also diminishes. At the same time, the contribution of bacteria that form butyrate increases. Selection for starch-using, butyrate-forming bacteria probably occurs during acarbose treatment because of the increased amounts of starch available for growth and fermentation in the colon.

The incorporation of less $^{13}CO_2$ into the methyl C than into the carboxyl C of acetate is probably due to the CO_2 exchange reaction catalyzed by the acetyl-SCoA synthase of the CO_2 reduction pathway (Fig. 1). Unlabeled formate is a possible product of carbohydrate fermentation by some of the microorganisms in the microbial community. This compound might be directly incorporated into the methyl group of acetate (Fig. 1). This would decrease the ratio of $^{13}CH_3$ to $^{13}COOH$. We examined the incorporation of [^{13}C]formate into the methyl group of acetate in the presence of unlabeled glucose and CO_2 by using specimens from 21 subjects (data not shown). The [^{13}C]formate was incorporated into the methyl group of acetate. However, since most of the formate was converted to $^{13}CO_2$, it was not possible to distinguish between direct incorporation and formation of the methyl group by reduction of CO_2.

Product labeling allowed us to compare the relative importance of the reduction of CO_2 to acetate or methane. Reduction of CO_2 to acetate diminished when methane was formed. The mean difference in the concentration of acetate formed from CO_2 between the methane-negative and methane-positive groups was 5 mM, and the mean concentration of methane formed by the positive group was 8 mM. This suggests that methanogens, when present, preferentially use electrons for reduction of CO_2 that are otherwise used by bacteria that reduce CO_2 to acetate. However, more acetate than methane was produced by CO_2 reduction by the flora of the methane-positive samples. The mean level of acetate formed from CO_2 by the methane-positive samples was 1.4 times greater than the mean level of methane formed from CO_2.

The labeling of propionate that occurred when [1-^{13}C]glucose was fermented showed that propionate was formed by the succinate pathway during all treatment periods. The labeling of both the C-2 and C-3 atoms of propionate by the C-1 atom of glucose is consistent with formation of the symmetrical succinate molecule after carboxylation of pyruvate and subsequent decarboxylation of succinate to propionate (Fig. 2). CO_2 incorporation into the carboxyl group of propionate (Table 2) is also consistent with the succinate pathway. However, the amount of CO_2 incorporated should be only 50% of the total amount of propionate carboxyl groups produced during fer-

mentation. Since this value was greater than 50% for the Aneg group, the results suggest that the enzymes of the colonic propionate-forming bacteria of this group catalyze the exchange of the C atoms of CO_2 and the carboxyl C of pyruvate (24).

The ^{13}C isotope distribution in all of the products is consistent with the use of the EMP pathway of glucose fermentation by the Aneg and Apos groups. CO_2 is not produced from C-1 of glucose by the EMP pathway (Fig. 2). The conversion of about 10% of the C-1 atoms to CO_2 may be due to a small amount of metabolism by other pathways in the colonic microbes. Formation of small amounts of CO_2 from C-1 of glucose may result from minor catabolic pathways that produce CO_2 from the C-1 position. This CO_2 may also result from anabolic pathways that generate pentose for biosynthetic reactions, as was concluded from observations made in a previous study in which a ^{14}C radioisotope analysis of the fecal fermentations of two healthy adults was performed (15).

Our results show that acarbose treatment results in decreases in the activities of colonic bacteria that use the Wood-Ljungdahl pathway and bacteria that form propionate and an increase in the activity of bacteria that produce butyrate. This is presumably caused by preferential growth of butyrate-forming bacteria after acarbose allows more starch to reach the colon. Measurements of overall fermentations and pathways by NMR or other isotope procedures can detect changes in the colonic fermentation that can be important to the host and are extremely difficult to ascertain by enumeration of colonic microbial species. For example, a twofold increase in the relative concentration of butyrate-forming species could increase the relative production of butyrate per day twofold. The magnitude of the bacterial concentration changes would be difficult to measure. However, doubling the supply of a major source of energy for colonic epithelial cells may be important to the host. The difficulty of applying microbial enumeration procedures to population changes in the colon may obscure important changes caused by drugs, diet, or large-bowel diseases that can influence host physiology and health. Fermentation product analyses and fermentation pathway investigations provide another approach for determining if significant changes in populations and fermentations are caused by specific agents or conditions.

ACKNOWLEDGMENTS

We thank Colette T. Tangel, Jean A. Krause, and Margaret M. Parfitt for assistance.

Portions of this study were supported by the National Institutes of Health, by National Cancer Institute grant CA56432, and by the Irving A. Hansen Memorial Foundation. Bayer Corporation provided acarbose and the placebo.

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