Digestibility of Selected Carbohydrates by Anaerobic Bacteria

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This paper describes the rationale by which nonnutritive food products may be developed and examines an in vitro fermentation model in which large numbers of compounds may be easily screened, early in their development, for their potential to be fermented in the human colon. Tests of anaerobic digestibility of selected carbohydrates by human fecal bacteria allowed the correlation of carbohydrate structure with susceptibility to the bacterial degradation. These tests also provided information about the noncaloric and physiological properties of carbohydrates. Forty-six mono-, oligo-, and polysaccharides were investigated. A novel group of nonmetabolizable carbohydrates, 5-*C*-(hydroxymethyl)hexoses, has been found. The critical parameter for the resistance to metabolism of these sugars appears to be branching at C-5 in the hexose molecule. 5-*C*-(Hydroxymethyl)hexoses are candidates for use in low-calorie foods as sucrose substitutes, nonnutritive bulking agents.

The objective of this work was to develop the methodology for discovering carbohydrates that could be used as nonnutritive sucrose substitutes in foods. Efforts of the food industry to provide sucrose-free foods and beverages have reflected the trend in industrialized societies toward reducing calorie intake. In beverages, high-intensity sweeteners (e.g., aspartame) can be used as a sucrose replacement in minute quantities since its primary functional goal is the sweet sucrose-like taste. On the other hand, nonnutritive sugar substitutes for foods have to be used in products in large quantities equivalent to the amount of sucrose, e.g. 30–40% of the total food mass. Consequently, they must be readily available and inexpensive and meet a number of very strict physicochemical and biological criteria.

A screening method for the development of nonnutritive compounds should contain three important elements: (1) clearly defined biological requirements or characteristics for a compound; (2) a procedure for selecting the test substances maximizing the probability of finding the desired characteristic; (3) a simple assay, based on relevant in vivo phenomena, allowing for high throughput screening. Among biological requirements, resistance to anaerobic digestion in the colon reduces the possibility of adverse physiological effects such as flatulence and diarrhea, while a lack of mammalian metabolism warrants nonnutritive properties. A simple and reliable in vitro broth fermentation assay using human fecal bacteria may provide a preliminary assessment of whether or not a compound is likely to be metabolized by the human colonic microflora in vivo. If a compound is fermented in a test tube, the possibility for its fermentation in the colon is increased and development of the compound as a major food constituent should proceed with caution. The human colonic microflora is a particularly interesting tool for investigating the resistance of carbohydrates to metabolism. It has a metabolizing power comparable to that of the liver for a broad range of substances (Drasar and Hill, 1974). Given this digestive potential, one could ask if the lack of anaerobic metabolism might also indicate the resistance of a carbohydrate to the mammalian metabolism and therefore hint nonnutritive properties.

A selection of carbohydrate structures that may be resistant to metabolism can be aided by examining metabolic pathways. The primary conversions of monosaccharides in the cells, nearly universal in biological systems, involve phosphorylation at the C-6 hydroxyl group and reactions at the anomeric center C-1. Moreover, the initial cleavage of oligo- and polysaccharides to simple sugars by glycosidases in the GI tract might be inhibited by the substituents at C-6 since oligosaccharides containing glucose 6-ethers are not substrates for glycosidases (Bock and Pedersen, 1987, 1988). Therefore, one could postulate that structural changes affecting reactivities at C-1 or C-6 may have a significant effect on the metabolism of carbohydrates in the human host and the human colonic microflora. To investigate how the characteristics of C-1 affect anaerobic metabolism, we have selected alditols of natural sugars, branched oligosaccharides and polysaccharides. In alditols, the character of C-1 is changed from carbonyl to alcohol. Branching in oligosaccharides introduces steric hindrance in the vicinity of C-1 which might limit access of glycosidases to the glycosidic bonds. One of the investigated polysaccharides, polydextrose, is a random glucose polymer used commercially as a bulking agent. Carbohydrates modified at C-5 and C-6 included 5-*C*-(hydroxymethyl)hexoses, L-glucose and water-soluble ethers of cellulose (Methocels). These modifications were expected to affect phosphorylation of C-6 and the susceptibility of oligosaccharides based on these sugars to glycosidases.

MATERIALS AND METHODS

Sources of Carbohydrates. Oligosaccharides **10**, **12**–**14**, and **23**–**28** (Tables III and IV) were synthesized by standard glycosidation [e.g., Paulsen (1982) and Mazur (1991)] using appropriately protected glycosyl acceptors, bromoacetyl sugars as the glycosyl donors, and mercuric cyanide in acetonitrile. Tetraglucosylsorbitol (**25**) and tetragalactosylsorbitol (**28**) were obtained by reduction of the corresponding pentasaccharides **24** and **27** with sodium borohydride. Synthesis of 5-*C*-hydroxymethyl sugars **11** and **32** through **44** (Tables III and VI; Figure 1) was based on the procedures described in the patent literature

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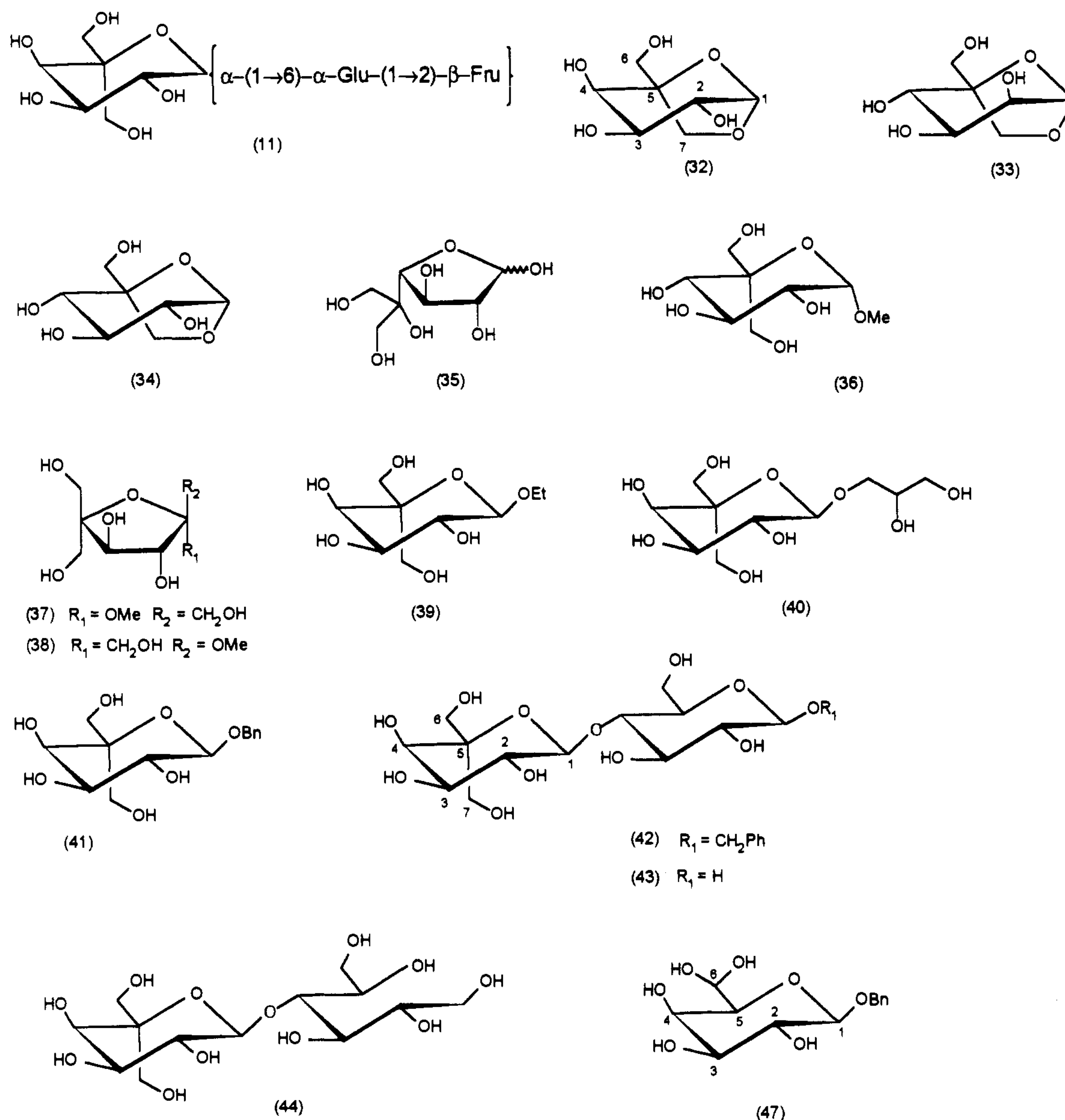

Figure 1. Structures of selected carbohydrates.

Table I. Representative ^{13}C NMR Characteristics of 5-*C*-(Hydroxymethyl)hexoses

compound	resonance frequency of hexose carbon atoms, ppm						
	C-1	C-2	C-3	C-4	C-5	C-6	C-7
benzyl β-D-*galacto*-hexadialdo-1,5-pyranoside (**47**)	101.7	71.4	72.7	68.2	76.8	88.3	
benzyl 5-*C*-(hydroxymethyl)-α-L-*arabino*-hexopyranoside (**41**)	98.9	71.1[a]	71.3[a]	68.9	80.0	61.4[a]	58.6[a]
1,6-anhydro-5-*C*-(hydroxymethyl)-L-altropyranose (**32**)	102.3	72.6	69.8	69.9	84.2	59.9	67.5

[a] Assignment may be reversed.

(Mazur, 1991). According to this method, the hydroxymethyl group is introduced at the hexose 5-carbon upon condensation of hexose 6-aldehyde with formaldehyde. The structures of **32** and **41** were confirmed by X-ray spectroscopy. Also, NMR spectroscopy and MS spectroscopy were used to confirm the structures of all carbohydrates prior to the digestibility tests. Table I shows representative changes in the resonance frequencies of the hexose ring carbon atoms taking place upon the transformation of 1,5-hexodialdoses to 5-*C*-(hydroxymethyl)hexoses. While the anhydro bridge in **32**–**34** could potentially be formed by either "upper" or "lower" hydroxymethyl groups, the latter configuration is thermodynamically more stable. It minimizes highly destabilizing 1,3-diaxial interactions (Cerny and Stanek, 1977) because two hydroxyl groups in **32** and **33** and all hydroxyls in **34** attached to the hexose ring are equatorial. If the upper hydroxymethyl group formed the anhydro linkage, these equatorial groups would become axial. Fructose oligosaccharide **15** was obtained by separation of Neosugar (Meji Seika Kaisha Co.) components. Other carbohydrates were obtained from commercial sources.

Human Feces. Fecal samples from five nonsmoking individuals in good health were used as the source of fecal inoculum. Because preparing freshly pooled feces from the five individuals for each fermentation run was not practical, i.e., an individual may become sick or begin medication which could lead to significant variations in fecal constituents, a homogeneous pool of frozen feces was used. In preliminary studies with 40 compounds, the fermentation profiles using an inoculum of freshly pooled feces were not significantly different from those in which

frozen pooled feces were used. Therefore, a frozen pool was used throughout the study. Fecal samples were collected in high-density sealable plastic bags (Uly-Pack Inc.), the air was squeezed out of the bags prior to sealing, and the bags were immediately frozen at −20 °C. To prepare pooled aliquots of feces for standard inocula, we placed the fecal samples together in a high-density sealable plastic bag flushed with argon and let it thaw. The feces were mixed by kneading, and 5-g aliquots were anaerobically dispensed into 50-mL culture tubes. The tubed 5-mL pooled fecal aliquots were sealed under argon and stored frozen at −80 °C until used for in vitro incubations.

Basal Medium. The basal medium used in this study is a modification of that used to study the fermentation of mucins and complex polysaccharides by pure cultures of intestinal bacteria (Wilkins and Walker, 1975; Vercellotti et al., 1978).

The basal medium consisted of 1.0% trypticase, 0.5% yeast extract, 0.5% sodium choloride, 0.00005% vitamin K, 0.01% phenol red, and 0.05% cysteine. The first two components were obtained from Difco Laboratories, Detroit, MI, and the latter four components were obtained from Sigma Chemical Co., St. Louis, MO. Control and experimental substrates were at a concentration of 0.5% in all experiments. Prereduced anaerobically sterilized (PRAS) basal medium was prepared according to standard methods (Holdeman et al., 1975) as 40-mL aliquots in 50-mL standard culture tubes and stoppered under anaerobic nitrogen. Individual selected carbohydrates (200 mg) were carefully weighed out onto sterile weighing paper and added to the 40-mL tubes of PRAS basal medium under 3% hydrogen, 10% carbon dioxide, and 87% nitrogen. The carbohydrate-supplemented basal broths were stoppered, mixed well, and kept for 2–3 h at ambient temperature prior to inoculation and incubation. Although anaerobes are often cultured under 100% CO_2, 10% CO_2 is recognized as being sufficient for the growth of human fecal anaerobes, which may be stimulated by CO_2, and is used often when solid, semisolid, and even liquid media are incubated anaerobically (Holdeman et al., 1975). In preliminary studies with 40 compounds, the fermentation data using 100% CO_2 were not significantly different from those in which 10% CO_2 was used. However, the variability, or the range of pH, was greater when 100% CO_2 was used. Therefore, 10% CO_2 was used throughout the study; H_2 was included to help mimic the colonic milieu.

Inoculation and Incubation. Standard inocula were prepared by thawing a 5-g tube of pooled fecal samples and adding to it 45 mL of prepared anaerobically sterilized PRAS dilution fluid. To minimize carry-over of fecal nutrients from the feces to the broth, we further diluted this preparation by adding 1 mL to 9 mL of PRAS dilution fluid. This standard inoculum thus represented a 1:100 dilution of the pooled feces.

At the anaerobic transfer device (Holdeman et al., 1975), each 40-mL tube of carbohydrate-supplemented basal broth was visually adjusted (as judged by the phenol red indicator) to pH 7.2–7.4 using sterile 0.25 M NaOH. To confirm a starting pH of 7.2–7.4, just prior to inoculation the pH of each supplemented broth was measured with an Accumet pH meter (Fisher Model 804) calibrated to 4.0 and 7.0. The pH-adjusted broth was then inoculated with 0.8 mL of the standard inoculum, stoppered under anaerobic gas (3% hydrogen, 10% carbon dioxide, and 87% nitrogen), and mixed well. Five-milliliter aliquots of each inoculated broth were dispensed into standard 25-mL culture tubes containing an inverted 0.5-mL glass vial, and the tubes were stoppered under anaerobic gas.

Table II. Fermentation of Controls during in Vitro Investigation of Selected Carbohydrates

	24 h		48 h		96 h	
control	pH	gas	pH	gas	pH	gas
basal medium	6.5	–	6.6	–	6.6	–
basal medium	6.6	–	6.6	–	6.7	–
glucose	4.7	++	4.7	+++	5.9	+++
glucose	4.6	++	4.8	+++	5.6	+++
starch	5.0	+++	5.3	+++	5.8	+++
starch	5.0	+++	5.3	+++	5.6	+++

For in vitro incubations, the broths were placed in a press (to avoid blown stoppers) and incubated at 37 °C for 24, 48, and 96 h. For each time period, glucose and starch broths as well as uninoculated broths were incubated as controls. For each incubation period, duplicate cultures containing test or control compounds were carefully removed from the incubation press, the gas formation was recorded, and the pH of each culture was determined using an Accumet pH meter (Fisher Model 804) calibrated to two points, 4.0 and 7.0.

Criteria for Fermentation. Substrates were classified as to whether or not they produced acids, gas, or both. A compound was fermented if the pH of the incubated broths was greater than 1.0 pH unit below the pH of uninoculated incubated basal controls. Fermentation was defined as "fast" if it occurred within 24 h; fermentation was defined as "slow" if it occurred within 24–96 h. The criteria for gas production was based on the amount of gas that displaced the fluid in the inverted glass tube: −, no gas production; +/−, borderline gas production (tube <20% full); +, minimal gas production (tube 20–80% full); ++, moderate gas production (tube 100% full); +++, maximal gas production (tube 100% full and under pressure). Although this is an imprecise means by which to measure gas formation, it is a classical way to rapidly determine whether gas is, or is not, produced in a large number of cultures. The data (pH/gas) for each compound at each time period are expresed as the average of duplicate tubes.

Absorption, Distribution, and Elimination Studies (ADE). The absorption, distribution, and elimination of aqueous solutions of either 1,6-anhydro-5-*C*-[^{14}C-hydroxymethyl]-L-altropyranose (**34**) or *O*-(5-*C*-([^{14}C]-hydroxymethyl)-α-L-*arabino*-hexopyranosyl)-D-sorbitol (**44**) were separately evaluated after a single oral dose to male Sprague–Dawley rats (n = 4/group). Animals were fitted with fecal cups and placed in individual stainless steel metabolism cages designed for the separation of urine, feces, and expired carbon dioxide. Rats were food-fasted for 16–8 h prior to and for 4 h after dosing. Purina rat chow was provided ad libitum for the remainder of the test period, and water was provided ad libitum throughout the study.

Rats (175–225 g) received 2 g/kg [^{14}C]-**32** (9 μCi) or 1.5 g/kg [^{14}C]-**44** (51 μCi) by gavage. At the end of the 72-h test period, the radioactive content of collected tissue and fluids was determined using a Beckman LS7800 liquid scintillation counter. Each sample was calibrated for efficiency using the automated external standard procedure of the instrument.

RESULTS

The results of the 96 h in vitro incubation time course for the selected carbohydrates are shown in Tables II–V. The data expressed for each time period for each compound represent an average value for duplicate tubes—values for all tubes were within 0.2 pH unit of each other.

Table II shows the average control values obtained for two representative in vitro incubation trials. The un-

Table III. Anaerobic Fermentability of Soluble Carbohydrates: Compounds Fermented Quickly[a]

entry no.		24 h		48 h		96 h	
		pH	gas	pH	gas	pH	gas
	monosaccharides						
1	D-sorbitol	5.6	+	4.8	++	4.7	+++
2	ethyl (α+β)-D-glucoside	5.0	++-	5.0	+++	5.0	+++
3	β-D-Gal*p*-(1→1)-glycerol	4.9	++-	4.6	+++	4.4	+++
	disaccharides						
4	sucrose	4.8	++	5.1	++	5.2	++
5	cellobiose	4.6	++	4.8	++	4.6	++
6	β-D-Glc*p*-(1→4)-D-sorbitol (cellobitol)	4.9	++-	4.9	+++	6.0	+++
7	lactose	4.7	++-	5.0	+++	4.9	+++
8	lactulose	4.5	+	4.6	++	4.6	++
9	β-D-Gal*p*-(1→4)-D-sorbitol (lactitol)	5.4	+	4.9	+	4.8	+
	trisaccharides						
10	4,6-di-*O*-β-D-galactosyl-(α+β)-D-glucose	5.4	++-	5.7	+++	6.0	+++
11	*O*-(5-*C*(hydroxymethyl)-β-L-*arabino*-hexopyranosyl)-(1→6)-α-D-Glc*p*-(1→2)-β-D-Fru*f* (raffinose derivative)	5.8	++	5.4	++	5.9	++
	tetrasaccharides						
12	cellotetraose	5.1	+	5.3	++	5.4	++
13	β-D-Glc*p*-(1→4)-β-D-Glc*p*-1→4)-β-D-Glc*q*-D-sorbitol	5.2	+	5.2	+	5.3	+
14	β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-D-Glc (lactotetraose)	5.4	++	5.7	++	6.1	+++
	pentasaccharides						
15	α-D-Glc*p*-(1→2)-β-D-Fru*f*-(1→2)-β-D-Fru*f*-(1→2)-β-D-Fru*f* (1→2)-D-Fru*f* (1-fructofuranosyl-nystose)	4.7	++-	4.8	+++	4.8	+++
	polysaccharides						
16	[→3)-β-D-Glc*p*-(1→]$_n$ (laminarin)	5.2	++	5.1	++	5.4	++

[a] Gas and ΔpH>1.0 unit of controls within 24 h.

Table IV. Anaerobic Fermentability of Soluble Carbohydrates: Compounds Fermented Slowly[a]

entry no.		24 h		48 h		96 h	
		pH	gas	pH	gas	pH	gas
	monosaccharides						
17	L-galactose	6.1	-	5.2-	+	5.1	+
18	methyl α-D-glucopyranoside	6.5	-	4.9	-	4.6	+/-
19	methyl (α+β)-D-fructofuranoside	6.3	+/-	5.3	++	4.9	++
20	1,6-anhydro-β-D-glucose	6.6	-	5.1	++	5.2	++
	disaccharides						
21	α-D-Glc*p*-(1→4)-D-sorbitol (maltitol)	6.5	+/-	5.3	+/-	5.0	++
22	α-D-Glc*p*-(1→6)-D-sorbitol	6.4	-	5.0	+	4.5	++
	trisaccharides						
23	*O*-β-D-galactopyranosyl-(1→4)-*O*-[β-D-galactopyranosyl-(1→6)]-D-sorbitol	6.0	+	5.5	++	5.9	++
	pentasaccharides						
24	2,3,4,6-tetra-*O*-β-glucopyranosyl-D-glucopyranoside	6.0	+/-	5.6	+	5.7	+
25	2,3,4,6-tetra-*O*-β-D-glucopyranosyl-D-sorbitol	5.7	+/-	5.6	+	5.9	+
26	ethyl 2,3,4,6-tetra-*O*-β-D-glucopyranosyl-(α+β)-D-glucopyranoside	5.9	+	5.4	+	5.7	++
27	2,3,4,6-tetra-*O*-β-D-galactopyranosyl-D-glucopyranose	6.4	-	6.2	+/-	5.6	+
28	2,3,4,6-tetra-*O*-β-D-galactopyranosyl-D-sorbitol	6.4	-	6.0	+	5.5	++
	polysaccharides						
29	polydextrose	6.0	+	5.7	+	5.9	+
30	arabinogalactan	6.1	+/-	5.8	+	5.7	+

[a] Gas and ΔpH > 1.0 unit of controls within 48–96 h.

supplemented basal broths decreased in pH to 6.5–6.7 during the 96-h incubation; this was possibly due to microbial production of acid products from the peptides and other organic components of the trypticase and yeast extract. The pH of these incubated basal cultures was the basis for setting the criteria pH for fermentation (see Materials and Methods). The positive control substrates, glucose and soluble starch, were readily fermented in 24–48 h, and after 96 h, the culture pH rose. This rise in pH over the 96-h incubation period was observed with a variety of substrates. This was possibly due to production of ammonia through secondary metabolism of acidic bacterial products or production of ammonia through the deamination of amino acids.

On the basis of the results of the in vitro fermentations, the test compounds can be placed into one of three metabolic groups. Tables III–V show the profiles for rapidly fermented, slowly fermented, and nonfermented test compounds, respectively. All of the compounds for which the character of the anomeric center (C-1 carbon) is the only structural variable are metabolized by the colonic flora, although at different relative rates. Highly branched oligo- (**23–28**) and polysaccharides (**29, 30**) (Table IV) exhibit consistently lower rates of metabolism than the linear tetra- (**12–14**), penta- (**15**), and polysaccharides (**16**) (Table III). Linear oligosaccharides and alditols such as disaccharides **4–9**, tetrasaccharides **12–14**, and pentasaccharide **15** are fermented at similar rates regardless of the chain length. Certain monosaccharides (**17–20**) and disaccharide alditols (**21, 22**) have been placed among compounds fermented slowly (Table IV) due to the lower initial rate of metabolism. However, the overall fermentation pattern for these sugars is not significantly different from that of compounds fermented quickly (Table III).

All carbohydrates modified at C-5 and C-6 (Table V; Figure 1) are not fermented. The lack of anaerobic metabolism has been shown for monosaccharides **32–35**, as well as simple glycosides **36–41**, and disaccharides **42–44**. On the other hand, longer linear 5-*C*-(hydroxymethyl)-hexose-based oligosaccharides with more than one common

Table V. Anaerobic Fermentability of Soluble Carbohydrates: Compounds Not Fermented[a]

entry no.		24 h		48 h		96 h	
		pH	gas	pH	gas	pH	gas
	monosaccharides						
31	L-glucose	6.6	–	6.6	–	6.6	–
32	1,6-anhydro-5-*C*-(hydroxymethyl)-L-altropyranose	6.6	–	6.7	–	6.7	–
33	1,6-anhydro-5-*C*-(hydroxymethyl)-β-L-gulopyranose	6.8	–	6.6	–	6.8	–
34	1,6-anhydro-5-*C*-(hydroxymethyl)-β-L-idopyranose	6.7	–	6.7	–	6.8	–
35	5-*C*-(hydroxymethyl)-(α+β)-L-*arabino*-hexofuranose	6.7	–	6.8	–	6.7	–
36	methyl 5-*C*-(hydroxymethyl)-α-D-*xylo*-hexopyranoside	6.8	–	6.6	–	7.0	–
37	methyl 5-*C*-(hydroxymethyl)-α-D-*erythro*-pentulofuranoside	6.7	–	6.7	–	6.8	–
38	methyl 5-*C*-(hydroxymethyl)-β-D-*erythro*-pentulofuranoside	6.7	–	6.8	–	6.7	–
39	ethyl 5-*C*-(hydroxymethyl)-α-D-*arabino*-hexopyranoside	6.7	–	6.7	–	6.7	–
40	(5-*C*-(hydroxymethyl)-α-L-*arabino*-hexopyranosyl)-(1→1)-glycerol	6.9	–	6.7	–	6.9	–
41	benzyl 5-*C*-(hydroxymethyl)-α-D-*arabino*-hexopyranoside	6.7	–	6.8	–	6.7	–
	disaccharides						
42	benzyl *O*-(5′-*C*-(hydroxymethyl)-α-L-*arabino*-hexopyranosyl)-(1→4)-β-D-glucopyranoside	6.9	–	6.7	–	6.9	–
43	*O*-(5-*C*-(hydroxymethyl)-α-L-*arabino*-hexopyranosyl)-(1→4)-(α+β)-D-glucopyranose	6.6	–	6.7	–	6.9	–
44	*O*-(5-(*C*-(hydroxymethyl)-α-L-*arabino*-hexopyranosyl)-D-sorbitol	6.8	–	6.7	–	6.8	–
	polysaccharides						
45	methocel E15	6.4	–	6.5	–	6.7	–
46	methocel A4M	6.4	–	6.4	–	6.7	–

[a] Gas and ΔpH within 0.5 unit of controls.

Table VI. Results of Oral Absorption, Distribution, and Elimination Studies[a]

parameter	compound	
	[^{14}C]-32	[^{14}C]-44
absorption	21.7 ± 0.7	3.0 ± 0.9
material balance		
feces	78.0 ● 1.3	98.7 ● 2.8
urine	21.0 ± 0.3	2.8 ± 0.9
carcass/tissue	0.5 ● 0.1	0.1 ± 0.0
expired CO_2	0.2 ± 0.0	0.1 ● 0.0
total	99.7 ± 1.4	101.5 ± 1.9

[a] All values are mean percentages ± SEM for four rats determined at 72 h after dosing. All percentages reflect dosed radioactivity.

hexose in the molecule such as derivatives of trisaccharide raffinose (**11**) (Table II) and modified arabinogalactan (not shown) are metabolized.

The results of ADE studies in rats conducted with [^{14}C]-**32** and [^{14}C]-**44** are shown in Table VI. Diarrhea was not observed in any animal over the 72-h test period. Feces was the major route of elimination for both compounds and contained 78.0% and 98.7% of the radioactivity from [^{14}C]-**32** and [^{14}C]-**44**-dosed animals, respectively. The mean ± SEM percent of absorbed radioactivity (sum of urine, carcass/tissue, and expired carbon dioxide radioactivity) from [^{14}C]-**32** was 21.7 ± 0.7% while that from [^{14}C]-**44** was 3.0 ± 0.9%. The majority of the absorbed radioactivity (93–97%) was eliminated in the urine with very little radioactivity retained in tissues or eliminated in expired carbon dioxide. This pattern of absorption, distribution, and elimination of [^{14}C]-**32** and [^{14}C]-**44** is very different from that of the digestible sugars, e.g., glucose, which undergo extensive absorption and catabolism.

DISCUSSION

The results indicate that, within the scope of modifications shown in this work, the character of the anomeric center (C-1) has no significant effect on metabolism. The only factor affecting the rate of metabolism of these compounds appears to be the branching of oligo- (**23–28**) and polysaccharides (**29, 30**) (Table IV). This suggests that the branching may hinder access of glycosidases to the glycosidic linkages.

In contrary, all modifications at C-5 and C-6 in the hexose molecule had a dramatic effect on metabolism. The presence of the additional hydroxymethyl group at C-5 appears to not only prevent degradation of the hexose backbone in compounds **32–35** but also inhibit cleavage of the glycosidic linkages in simple glycosides **36–41**, and in disaccharides **42–44**. On the other hand, this protection does not extend beyond the sugar directly linked to 5-*C*-(hydroxymethyl)hexose. Thus, analogs of raffinose (**11**) (Table III) and arabinogalactan (not shown) have approximately one 5-*C*-(hydroxymethyl)hexose unit for every two molecules of the natural sugar (mostly galactose), and they are metabolized. It should be pointed out that our results are not sufficient to extrapolate the lack of anaerobic metabolism on hexoses having the additional substituents at C-5 different from the hydroxymethyl group. The metabolic properties of new analogs should be determined experimentally.

Theoretically, one could consider two possible explanations for the lack of metabolism among 5-*C*-(hydroxymethyl)hexose derivatives. One reason might be the presence of a 5-*C*-hydroxymethyl group in the stereochemical configuration found in L-hexoses. With the exception of L-galactose (**17**) (Pigman and Horton, 1972) (Table IV) and recently discovered L-altrose (Stack, 1987) (not investigated), other L-aldohexoses have not been found in nature. Consequently, bacterial enzymes may not be able to effectively catalyze reactions involving many L-hexoses, as shown in the case of L-glucose (**31**) (Table V). This effect could also be reflected in the lack of metabolism of 5-*C*-(hydroxymethyl)hexoses. Another possibility is that a specific configuration of the 5-*C*-hydroxymethyl group is not critical but that branching at C-5 creates a barrier for the access of bacterial enzymes to these substrates.

In an attempt to evaluate the importance of each factor, metabolism of the 5-*C*-hydroxymethyl derivatives **37** and **38** was investigated. Glycosides **37** and **38** can be regarded as being derivatives of D-fructose or L-sorbose. The two latter compounds are metabolizable natural sugars (Krieg and Holt, 1984) which differ only in the configuration of their hydroxymethyl group at C-5. If only configuration but not branching at C-5 was important for the metabolism, both **37** and **38** should be digested since otherwise they have all the structural elements of digestible sugars. The results show that compounds **37** and **38** are not metabolized, which indicates that the branching at C-5 in 5-*C*-

(hydroxymethyl)hexoses may be the critical parameter for the resistance to metabolism.

The adequacy of the in vitro screening method can be judged from the in vivo properties of the selected leads. The ADE results summarized in Table V suggest that 5-*C*-(hydroxymethyl)hexoses may indeed be resistant to the metabolism in vivo as was predicted from the in vitro fermentation experiments. On the basis of these results, we consider 5-*C*-(hydroxymethyl)hexoses to be promising candidates for bulking agents. In addition to their noncaloric properties, the resistance of these compounds to digestion by the colonic microflora should lower the possibility of flatulence and diarrhea upon consumption, compared to known digestible and partially digestible bulking agents such as polydextrose. Although the reported in vitro model appeared to predict nonmetabolism in vivo, further experiments comparing in vitro and in vivo metabolism of a larger number of samples are needed to rule out a coincidental correlation. In addition, only through adaptation studies, such as serial transferring of a compound into supplemented broths over time, can the fermentative potential for a compound involved in long human exposure be properly determined.

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