Table IV. Number of Monomer Sugar Units in a Carbohydrate Oligomer Calculated from Theoretical Values of P/D

P/D	no. sugar monomers	P/D	no. sugar monomers
0.0	1	0.167	7
1.0	2	0.143	8
0.5	3	0.125	9
0.33	4	0.111	10
0.25	5	0.071	15
0.20	6	0.053	20

curve, in either slope or intercept.

Measurement of Unknowns. A satisfactory analysis of end groups by the present method should be possible for most unknown samples. As mentioned, each lot of $NaBD_4$ must be tested by reduction of a known sample to determine the extent of deuterium incorporation. Also, there must be no interference from the sample in the mass region of interest. Interference is minimized by capillary chromatography and by restricted data acquisition at a relatively high ratio of ion mass to charge. If there is a suspicion of impurity or background interference, the sample can be tested by performing both reductions on a portion of the sample with sodium borodeuteride and both reductions on a second portion with sodium borohydride. The preferred sequence is first to reduce the oligomer reducing end with borohydride and, after hydrolysis, to reduce with borodeuteride. This method produces less protio than deuterio material and lessens the correction for protiated fragments containing the ^{13}C isotope (the M′ + 1 peak).

We tested the analytical method on several samples of loblolly pine kraft pulp that had been treated with polysaccharidases. The resultant "enzyme" lignin contained oligomeric fragments of wood polysaccharides. The results for these samples are presented in Table III. Calculated amounts and their standard deviations were determined from the unweighted data of Table IIB. An apparent number average degree of polymerization ($\overline{DP}_n$) can be calculated from these data by $\overline{DP}_n = D/P + 1$. Further interpretation depends on how much is known about the original polysaccharide structures. To convert sugar analysis results to percent of the original samples, appropriate factors must be included for hydrolysis losses and anhydride structures as well as the molecular weight factors resulting from derivatization. Usually end group and sugar analyses must be combined with other structure determination techniques, such as methylation analyses, for complete interpretation.

CONCLUSION

The residual oligosaccharidic fragments present in "enzyme lignins" after enzymatic hydrolysis of wood polysaccharides have a very low $\overline{DP}_n$ and are ideally suited to the present type of analysis. For $\overline{DP}_n$ = 8–9 or less, the analysis is accurate to one monomer unit (Table IV). Above that, the ratio difference due to a change of one monomer unit is equal to or smaller than the standard deviation for the calculated ratio (Table III).

Registry No. $NaBH_4$, 16940-66-2; $NaBD_4$, 15681-89-7; arabinose, 147-81-9; xylose, 58-86-6; mannose, 3458-28-4; glucose, 50-99-7; galactose, 59-23-4.

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Caloric Utilization of Sorbitol and Isomalt in the Rat

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Sorbitol and isomalt are modified saccharides used to substitute for the physical properties of sucrose in various prepared foods. The merits of the various methods for determining caloric availability were reviewed. Balance and growth curve methods are inaccurate and inappropriate for determination of the caloric availability of these substances when present in diets at low concentrations, whereas the radiolabel disposition method is a direct and precise measure of utilization. Accordingly, we administered uniformly ^{14}C-labeled material to rats and collected excreta and expired air. The appearance of about half of the label in CO_2 indicated that, by comparison with labeled glucose, about 80% of the orally administered sorbitol and isomalt was calorically available to the rat. The high caloric availabilities of these materials were confirmed by the appearance in feces of only 14 and 12% of the administered label from sorbitol and isomalt, respectively.

In addition to providing essential nutrients, the food we consume supplies energy for physiological maintenance and growth. To avoid the undesired weight gain resulting from energy intake beyond these needs, high-intensity sweeteners are gaining popularity as a replacement for sucrose. Modified saccharides (bulking agents) have also been developed to provide the texture and bulk necessary in many prepared foods but not provided by high-intensity sweeteners alone. These modified saccharides can replace the bulk of sucrose, and sometimes that of other ingredients, approximately on a 1:1 (w/w) basis. Because they

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are used only in selected foods, they are typically present in low concentrations (less than 10%) in the total diet. To estimate the caloric value of these saccharides, several test methods have been employed; these include balance studies, growth trials, and radiolabel disposition methods. For various reasons these methods are not equally useful for determining the caloric value of modified saccharides and, in fact, some can lead to entirely erroneous conclusions.

Balance studies involve the substitution of a test substance into a highly digestible basal diet. Energy potentially available to the host as digestible energy (DE, the energy of the diet minus fecal energy) or metabolizable energy (ME, diet minus fecal and urine energy) is quantitated in the basal and test diet. The caloric value of the test substance is then quantitated from caloric differences (Church and Ford, 1974; Mitchell, 1964). The desirability of using high levels of the test substance [35–100% of the daily intake in studies used to generate human tabulated caloric values (Merrill and Watt, 1973)] makes the balance method inappropriate for determining the caloric values of bulking agents. Even when the most rigorous dietary and environmental controls, necessary in all balance trials (Lentner et al., 1975; Young, 1986) are exercised, there are appreciable experimental errors associated with the balance method. The procedures for analyzing the nutrient composition of the food and fecal material are inexact (Merrill and Watt, 1973) and food intake and fecal excretion data have errors associated with them (Hegsted, 1975). The standard error of the mean was 2–8% in recent well-controlled animal studies (Krishnamachar and Canolty, 1986; Rothwell and Stock, 1984; Dulloo et al., 1985; Deb et al., 1975) and 3–10% in human studies (Goranzon et al., 1983; Calloway and Kretsch, 1978; Calloway and Chenoweth, 1973; Southgate and Durnin, 1970; Norgan and Durnin, 1980). When the test food is a bulking agent that is limited to 10% of the diet, even small variations in the measured total caloric value will cause the confidence intervals associated with the calculated caloric value of the saccharide to be so broad that the result will be meaningless (Pesti and Ware, 1986).

The balance method quantitates ME as the difference between intake and excretion in feces and urine. While this is generally appropriate for well-digested foods, it is not an accurate measure of energy delivered to the animal by these modified saccharides. Fermentation, primarily to absorbed short-chain fatty acids, can be a major route for the delivery of energy from these substances. Although the fatty acids formed are correctly accounted for in the balance method, the methane and hydrogen also resulting from fermentation are not collected in the "excreta" and incorrectly appear as energy delivered to the animal. Similarly, the increased bacterial mass in feces is correctly accounted for, but the additional substrate consumed (the "cost" of the formation of additional bacteria) and appearing in flatus as CO_2 is not calculated as part of the energy unavailable to the animal (Czerkawski and Breckenridge, 1969).

Growth trials include measurement of weight gain during a period of feeding a test food substituted for a portion of a basal diet. Comparisons are made in weight gain in response to the test food vs. the reference basal diet or a highly digestible reference standard, such as sucrose. The control diets are fed concurrently to a group of similar animals (Church and Ford, 1974; Mitchell, 1964). Environmental conditions must be rigorously controlled; e.g., a difference of 4 °C in room temperature can result in a 34% difference in weight gain (Rice et al., 1957).

The limitation in dose tolerance in growth trials is the same as in balance trials for bulking agents. Since only a small fraction of the diet can be replaced, broad confidence intervals prevent the ability to reliably quantitate a treatment difference. In addition, changes in weight may reflect only changes in intestinal contents caused, for example, by water retention due to presence of the bulking agent in the intestinal lumen. The caloric cost of energy disposition varies according to the type of tissue synthesized [i.e., protein, fat, bone (Just, 1984)]. Even in growing animals, energy disposition will vary while absolute weight changes may be similar (Goodhart and Shils, 1980; Hill and Anderson, 1957). Also, net energy values vary with the age and the physiological state of the animal. The net energy for maintenance is not the same as for growth (Mitchell, 1964; Just, 1984; Goodhart and Shils, 1980; Hill and Anderson, 1957; Blaxter, 1971). Therefore, caloric utilization determined by necessity in young rapidly growing animals will have little applicability for grown animals.

The caloric utilization of bulking agents can also be determined by the disposition method. In this procedure a uniformly ^{13}C- or ^{14}C-labeled test substance is administered at a dose similar to that projected for human use. Excretion of labeled material in feces, urine, and exhaled CO_2 is measured. Quantification of the label in the feces will give accurate values for the portion of the test material unavailable for absorption. Expired CO_2 represents nutrients delivered directly to the host and after the fermentative action of intestinal bacteria. A portion of the nutrients made available to the host (by direct means or after fermentation to fatty acids by the intestinal bacteria) is not metabolized to CO_2 and exhaled over the duration of the experiment but is incorporated into tissue. Reliable correction factors, based on exhaled CO_2 after administration of fully absorbed reference compounds such as glucose and fatty acids, will correct for this. The intestinal bacteria also generate CO_2 directly; some of this CO_2 is absorbed by the host and exhaled, and the remainder is expelled as flatus. In the disposition method, all of the dose can be accounted for. Label in feces and flatus represents calories not provided to the host; part of the label in expired CO_2 represents calories made available (directly and indirectly) to the host, while part represents calories provided to the intestinal bacteria.

The disposition method has several advantages over the balance and growth curve methods. It is a direct measure of the test substance in question. The disposition method, since it examines only the test substance and its products by a highly sensitive technique, can be employed at doses reflective of human use. Unlike the growth curve method, it can be used in adult animals. Of greatest relevance, the disposition method can be used in humans, impossible for the growth curve method and virtually impossible for the caloric balance method. Although animal models are useful, particularly in preliminary experiments, greatest reliance should be placed on methods that can also be applied to humans.

In our continuing effort to accurately evaluate the caloric density of various sucrose replacements, we have used the radiolabeled disposition method to determine the caloric availability of polydextrose in rat (Figdor and Rennhard, 1981) and man (Figdor and Bianchine, 1983) and the availability of maltitol in rat, dog, and man (Rennhard and Bianchine, 1976). We continue these investigations with sorbitol and isomalt (Palatinit) in this report.

Sorbitol, a monosaccharide with mixed claims for reduced caloric content, has achieved widespread use in the

food industry, since it combines acceptable sweetness with reduced cariogenicity (Shaw, 1976) and insulin demand (Steinke et al., 1961). The results here indicate that after administration to rats sorbitol has approximately 80% of the calories of glucose. It is utilized primarily by absorption from the upper gastrointestinal tract but also by fermentation in the lower gut. Isomalt, an equimolar mixture of the disaccharides α-D-glucopyranosido-1,6-sorbitol and α-D-glucopyranosido-1,6-mannitol is currently under evaluation for use as a sugar substitute in prepared foods. Although the sweetness of isomalt is reportedly approximately half that of sucrose, the claimed caloric reduction is 30–60% that of sucrose (Grupp and Siebert, 1978). In rat experiments, we now show that isomalt supplies about 80% of the calories of glucose; it is utilized mainly by absorption of the hydrolyzed components from the upper gastrointestinal tract.

METHODS

Uniformly labeled [^{14}C]-D-sorbitol was purchased from New England Nuclear.

Uniformly labeled [^{14}C]isomalt was prepared from uniformly labeled [^{14}C]sucrose (New England Nuclear). Sucrose (2 g, 10 mCi) was converted to isomaltulose (Schiweck, 1975), which was subsequently reduced with Raney nickel to obtain isomalt (Schiweck et al., 1978). Isomalt was purified and characterized essentially as described by Schiweck (1980). Additional purification was carried out by chromatography on Bio-Gel P2 (Bio–Rad Laboratories, Richmond, CA). The [^{14}C]isomalt so obtained was identical with an authentic sample of isomalt in all respects when compared by infrared spectra, NMR spectra, optical rotation, gas–liquid chromatography (of the persilylated derivatives), and thin-layer chromatography. The specific activity of the [^{14}C]isomalt was 3.13 μCi/mg. To ensure that the [^{14}C]isomalt was uniformly labeled, a sample was hydrolyzed with hydrochloric acid, and the isomalt components (glucose, mannitol, sorbitol) were isolated and purified. The specific activities of the recovered glucose, mannitol, and sorbitol were identical, and the total radioactivity recovered in the sorbitol fraction was identical with that obtained from the mannitol fraction. As expected, the radioactivity recovered in the glucose fraction was twice that of either the mannitol or sorbitol.

Fasted male Sprague–Dawley rats weighing approximately 200 g each were used throughout these experiments. Test substances were administered by gavage in 1 mL of water. The glucose-treated rat received 12.9 μCi (100 mg/kg). Isomalt-treated rats received 12.5 μCi per rat (100 mg/kg), and the sorbitol-treated rats received 10.5 μCi (50 mg/kg). Intravenously administered sorbitol was given in 0.5 mL of water via the tail vein (5.5 μCi, 25 mg/kg).

The $^{14}CO_2$ collection procedure, urine and fecal assays, and counting methods have been described (Figdor and Rennhard, 1981). $^{14}CO_2$ was collected at hourly intervals for a total of 13 h.

RESULTS

The recovery of radioactivity from rats after oral administration of [^{14}C]sorbitol is summarized in Table I. An average of 48.0% of the administered radioactivity was recovered as $^{14}CO_2$ during the 13-h collection period. Application of the established catabolic conversion factor of 0.6 (Figdor and Bianchine, 1983; Figdor and Rennhard, 1981; Wursch et al., 1979) indicates that orally administered sorbitol has 80% of the calories of glucose. The time course of expired $^{14}CO_2$ from rats is shown in Figure 1. The remainder of the administered radioactivity was recovered in urine (2.6%) and feces (14.2%).

Table I. Administration of [^{14}C]-D-Sorbitol

	% administered dose recovered			
	oral			
collection hours	rat 1	rat 2	mean	iv
$^{14}CO_2$				
1	0.21	0.12	0.17	8.92
2	1.24	0.67	0.96	10.94
3	6.20	1.82	3.46	6.85
4	9.48	9.65	9.75	54.14
5	8.63	7.11	7.87	2.68
6	5.62	7.15	6.39	1.82
7	3.94	9.43	6.69	1.45
8	2.65	4.18	3.42	1.06
9	2.01	3.41	2.71	0.83
10	2.49	2.51	2.56	0.70
11	1.82	1.96	1.89	0.55
12	1.26	1.44	1.35	0.46
13	1.01	0.99	1.00	0.49
total $^{14}CO_2$	45.5	50.4	48.0	40.9
caloric utilizn	75.9	84.1	79.9	68.2
urine				
0–24	2.30	2.30	2.30	14.50
24–48	0.21	0.17	0.19	0.26
48–72	0.13	0.09	0.11	0.17
total urine	2.64	2.56	2.60	14.39
feces				
0–24	10.98	8.99	9.99	4.26
24–48	2.83	3.20	3.02	1.02
48–72	1.21	1.07	1.14	0.52
total feces	15.02	13.26	14.15	5.79
total: caloric utilizn + urine + feces	93.4	99.9	96.7	88.9

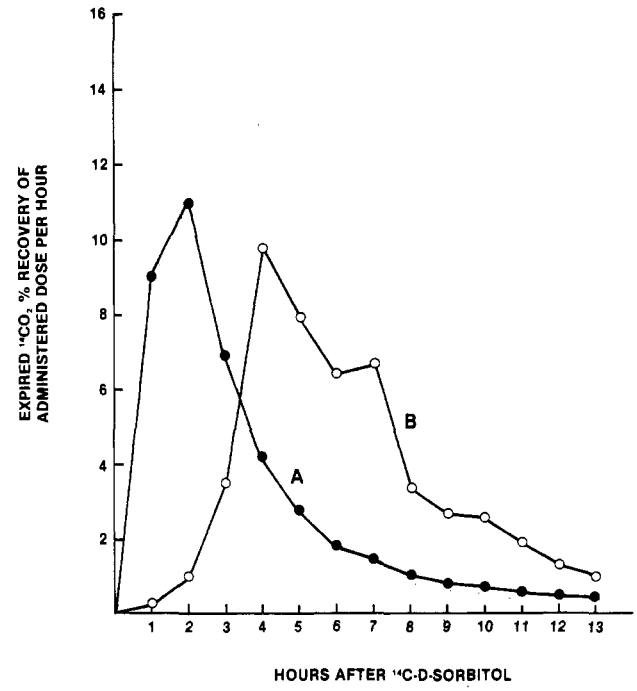

Figure 1. Recovery of expired $^{14}CO_2$ from the rat after a single dose of [^{14}C]sorbitol: A, intravenous; B, oral.

After intravenous administration of [^{14}C]sorbitol 40.9% of the administered radioactivity was recovered in the breath, indicating a caloric utilization of 68%. The time course of expired $^{14}CO_2$ also differs significantly from that seen after oral administration, with more radioactivity obtained during the first 2 h of collection. As expected, more radioactivity was excreted in urine and less in feces after iv administration than after oral administration (Table I; Figure 1).

The recovery of radioactivity from rats after oral administration of uniformly labeled [^{14}C]isomalt is summarized in Table II. An average of 48.1% of the administered

Table II. Oral Administration of [^{14}C]Isomalt

	% administered dose recovered					
collection hours	rat 1	rat 2	rat 3	rat 4	mean	SD
$^{14}CO_2$						
1	3.68	3.77	2.40	2.38	3.06	0.8
2	7.94	7.19	7.21	7.17	7.39	0.4
3	15.14	5.80	5.50	12.60	9.76	4.9
4	9.34	9.19	5.57	7.68	7.95	4.9
5	4.87	8.92	5.72	4.65	6.04	2.0
6	3.14	5.34	3.39	2.55	3.61	1.2
7	1.86	3.69	4.15	2.10	2.95	1.1
8	1.38	2.36	3.25	1.30	2.07	0.9
9	1.00	1.67	2.74	1.03	1.61	0.8
10	0.78	1.37	1.86	0.82	1.21	0.5
11	0.64	1.00	1.43	0.71	0.95	0.4
12	0.53	0.93	1.17	0.59	0.81	0.3
13	0.45	0.66	0.88	0.55	0.64	0.2
total $^{14}CO_2$	50.8	51.9	45.3	44.2	48.1	3.9
caloric utilizn	84.7	86.5	75.5	73.6	80.1	6.4
urine						
0–24	1.52	2.40	8.3	0.91	3.2	3.3
24–48	0.42	0.12	0.33	0.18	0.3	0.1
48–72	0.02	0.12	0.07	0.19	0.1	0.1
total urine	1.96	2.64	8.53	1.28	3.6	3.3
feces						
0–24	11.85	4.49	3.92	13.76	8.5	5.0
24–48	2.31	1.19	1.35	7.65	3.1	3.1
48–72	0.55	0.49	0.31	0.53	0.5	0.1
total feces	14.71	6.17	5.58	21.94	12.1	7.8
total: caloric utilizn + urine + feces	101.3	95.3	89.6	96.9	95.8	4.8

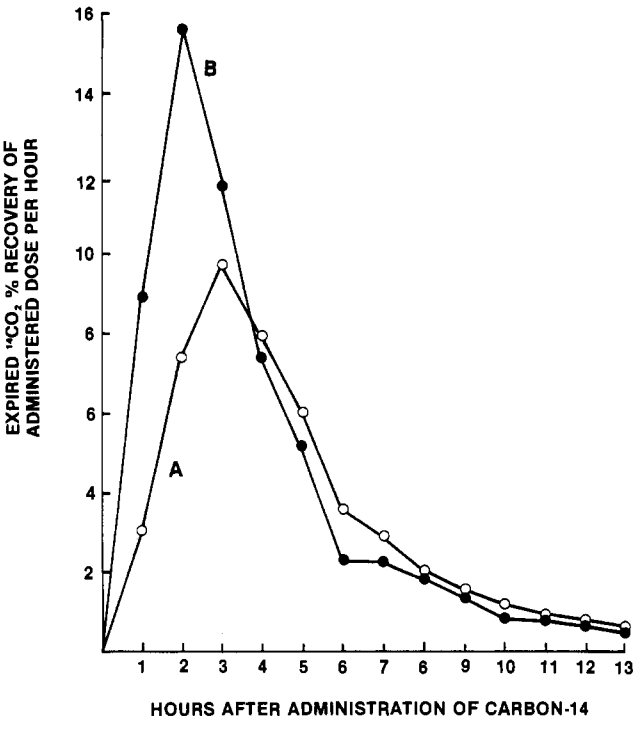

Figure 2. Recovery of expired $^{14}CO_2$ from the rat after a single oral dose of [^{14}C]isomalt (A) or [^{14}C]glucose (B).

label was recovered as expired $^{14}CO_2$ during the 13-h collection period, indicating a caloric utilization of 80% for isomalt. The maximum rate of recovery of $^{14}CO_2$ was during the third hour of collection (Figure 2). Urinary recovery of radioactivity was 3.6% and fecal recovery was 12.1% for a total recovery of 95.8% of the administered radioactivity. The time course of the exhaled $^{14}CO_2$ from the four rats that received [^{14}C]isomalt is shown in Figure 2. For reference, Figure 2 also shows the time course of exhaled $^{14}CO_2$ obtained from a rat after oral [^{14}C]-D-glucose (uniformly labeled) administration.

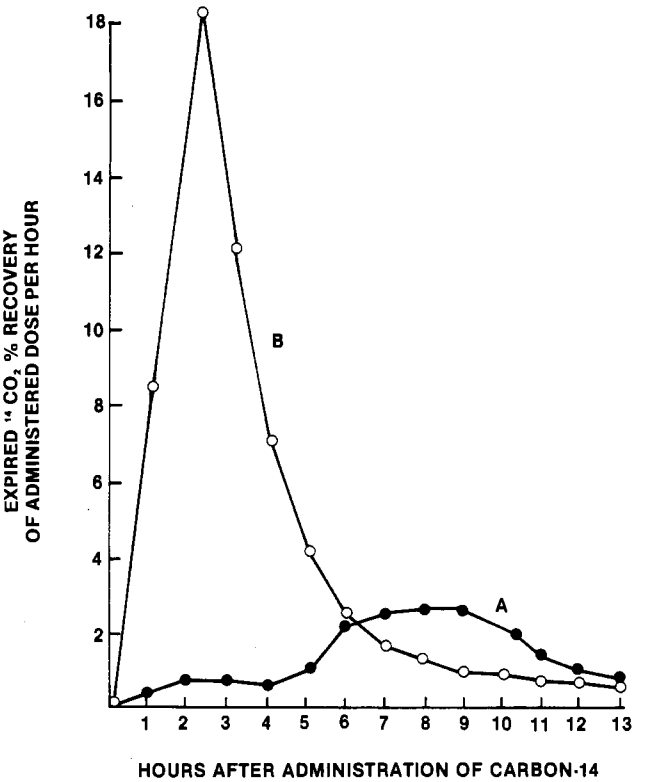

Figure 3. Recovery of expired $^{14}CO_2$ from the rat after a single oral dose of [^{14}C]polydextrose (A) or [^{14}C]glucose (B).

DISCUSSION

Readily absorbed monosaccharides are rapidly metabolized by mammalian enzymes, largely converted to CO_2, and exhaled in the breath. Glucose is an appropriate example; the pattern of expired $^{14}CO_2$ from the rat after oral administration of [^{14}C]-D-glucose (uniformly labeled) is shown in Figure 2. Maximum exhalation of $^{14}CO_2$ occurs during the second hour of collection, and the total $^{14}CO_2$ recovered during the 13-h collection interval is approximately 60% of the administered radioactivity (Figdor and Rennhard, 1981). The remainder is converted to glycogen and other tissue components and eventually also metabolized to CO_2. Those saccharides that are less well absorbed from the upper gastrointestinal tract may eventually undergo fermentation by the microorganisms of the lower GI tract and be converted to short-chain fatty acids. Some of these are absorbed and ultimately yield CO_2, which is also removed via breath (Cummings, 1983). An example is polydextrose, a low caloric bulking agent that is not absorbed and only partially degraded by the gut microflora (Figdor and Rennhard, 1981; Figdor and Bianchine, 1983). The pattern of expired $^{14}CO_2$ recovered from the rat after oral administration of uniformly labeled [^{14}C]polydextrose is shown in Figure 3 A shift in the CO_2 recovery curve to later times is indicative of less digestive hydrolysis and more bacterial fermentation. In comparison to glucose, only 15% of the polydextrose is expired as CO_2; this represents the combined CO_2 production from direct host utilization and bacterial fermentation, suggesting that the maximal caloric utilization of polydextrose is 25% that of glucose. The recovery of more than half of this bulking agent intact in feces clearly indicates that the bacterial flora are incapable of fully converting such a polymer to absorbable monomers during its residence time in the gut. It is not clear whether the fraction metabolized represents only the lower molecular weight fractions or whether there is also some hydrolysis of susceptible glycosidic linkages on the "periphery" of the higher molecular weight fractions.

The similarity in results obtained in rats vs. humans for several compounds suggests that the rat is an appropriate species in which to assess the caloric availability of additional agents. The time course of evolution of labeled CO_2, which provides information on the extent of fermentation, also appears to be similar in the two species. Growth curve and balance studies have been conducted primarily in rats, further supporting the use of this species in radiolabel disposition studies. Finally, studies in the rat require a minimum of labeled compound, and the evolved CO_2 can be readily collected. Accordingly, the rat was selected for our studies with sorbitol and isomalt.

The absorption, utilization, and disposition of sorbitol and mannitol have been addressed in a series of earlier reports. Both polyols are incompletely absorbed from the upper intestinal tract, and that absorbed is degraded by mammalian enzymes. That which escapes absorption is fermented by the intestinal microflora of the lower digestive tract. A major distinction between these two hexitols is that mannitol is less well absorbed than sorbitol. In addition, the utilization of mannitol is less than that of sorbitol since it is more rapidly excreted intact. When Smith et al. (1940) administered sorbitol or mannitol intravenously to man, 85% of the mannitol was recovered in urine but only 32% of the sorbitol.

With ^{14}C-labeled glucose and sorbitol it was shown that 1 h after oral administration 97% of the glucose but only 75% of the sorbitol was absorbed (Wick et al., 1951). After intravenous administration, only 3% of the administered label from glucose was recovered in the urine but 25% of the label from sorbitol was found in urine (Keller and Froesch, 1971).

Schell-Dompert and Siebert (1980) showed that maximum respiratory $^{14}CO_2$ occurred in rats 2 h after intravenous [^{14}C]sorbitol administration and 5 h after oral [^{14}C]sorbitol administration. They concluded that sorbitol is only partially absorbed from the small intestine and undergoes fermentation in the lower part of the digestive tract. These authors demonstrated the presence of large amounts of hydrogen in the breath of humans that received nonlabeled sorbitol, further suggesting fermentation of this polyol. More recently, in tests connected with the treatment of alcoholic cirrhosis, McClain et al. (1981) concluded that sorbitol is metabolized by man in large part by gut bacteria.

Our own studies confirm and expand upon these observations. Intravenously administered [^{14}C]sorbitol is rapidly and effectively metabolized by the rat, and most of the $^{14}CO_2$ is expired during the first 2 h following injection. Approximately 15% of the administered radioactivity is excreted in urine. The caloric utilization of sorbitol under these conditions is 68%. After oral administration of [^{14}C]sorbitol to the rat, maximum exhalation of $^{14}CO_2$ occurs at 4 h, with the $^{14}CO_2$ recovery curve shifted to the later times indicative of some fermentation. Urinary excretion of radioactivity after oral administration is only 2.5% of the administered dose. Under these conditions, the caloric utilization of sorbitol has increased to 80% (Table I; Figure 1).

Mannitol, on the other hand, is so rapidly excreted after injection that it is difficult to detect any caloric utilization. After oral administration, however, intestinal fermentation causes significant utilization of mannitol. After intraperitoneal injection of [^{14}C]mannitol to the rat, Wick et al. (1954) showed that 2–3% of the administered radioactivity was recovered as $^{14}CO_2$, and 85% of the dose was recovered in urine. That mannitol is indeed metabolized by mammalian enzyme systems was demonstrated by direct injection into the portal system followed by the recovery of 71% of the administered [^{14}C]mannitol as exhaled $^{14}CO_2$. After oral administration of [^{14}C]mannitol to the rat 29% of the administered label was recovered as $^{14}CO_2$, indicating a caloric utilization of 48%. Very little exhaled $^{14}CO_2$ was collected during the 3-h interval after mannitol administration; most of the $^{14}CO_2$ was obtained during the later collection intervals, indicating that fermentation accounted for most of the $^{14}CO_2$. Nasrallah and Iber (1969) demonstrated that, after intravenous administration of [^{14}C]mannitol to man, only 2.5% of the label was recovered as $^{14}CO_2$, and most of the ^{14}C was found in urine and feces. After oral administration approximately 19% of the dose was recovered as exhaled $^{14}CO_2$ during the 12-h collection period. Maximum $^{14}CO_2$ expiration occurred during the interval of 9–10 h after [^{14}C]mannitol administration.

Table III. Calculated Caloric Utilization of Isomalt

saccharide	caloric utilizn
2 × glucose at 100%	200%
sorbitol	80
mannitol[a]	48
av caloric utilizn	82%

[a] The caloric utilization for mannitol is taken from the rat studies of Wick et al. (1954).

The disposition and metabolism of isomalt have been described by Grupp and Siebert (1978). Isomalt is cleaved enzymatically by maltase (α-glucosidase) of jejunal mucosa, liver lysosomes, and yeast. That some isomalt escapes this digestion process was demonstrated by finding small quantities of the constituent parts of isomalt, glucopyranosidosorbitol (GPS), and glucopyranosidomannitol (GPM) as well as the split hexitols sorbitol and mannitol, in the caecum and large intestine of rats up to 6 h after isomalt feeding. At no time was any glucose found in the intestinal tract, and it was assumed that any glucose so formed was rapidly absorbed. Since less than 1% of the orally administered isomalt was recovered in the feces as GPS, GPM, sorbitol, or mannitol, it was concluded that any isomalt or its parts that escape digestion and absorption from the upper GI tract must be efficiently degraded via bacterial fermentation in the lower GI tract.

The results presented in this report confirm the above observations and also permit quantitation of the utilization of isomalt. Recovery of exhaled $^{14}CO_2$ after [^{14}C]isomalt administration indicates that a major fraction of isomalt is rapidly utilized via hydrolysis and absorption from the upper gastrointestinal tract followed by mammalian enzymatic catabolism (Table II; Figure 2). The shift of the isomalt $^{14}CO_2$ exhalation curve to the later times (relative to glucose) is undoubtedly due to the slow hydrolysis of part of the isomalt, as reported by Grupp and Siebert (1978), as well as some contribution from the intestinal fermentation of isomalt and/or its components. The net sum of these processes indicates a caloric utilization that is approximately 80% of that for glucose.

Since it has been shown that isomalt is cleaved by the jejunal mucosa, albeit slowly (Grupp and Siebert, 1978), the caloric utilization of isomalt should be equal to the sum of its constituent parts sorbitol, mannitol, and glucose. The calculated caloric utilization of isomalt of 82% is in good agreement with the experimentally derived value of 80% (Table III).

Although these studies with sorbitol and isomalt and earlier studies with other agents employed single doses, caloric availability on repeated administration must be addressed. Alterations in levels of volatile fatty acids in

the intestine of weanling rats have suggested that the bacterial flora may adapt to the presence of a bulking agent and more fully convert it to absorbable nutrients upon prolonged exposure (Grossklaus et al., 1984). However, fatty acids were quantitated only at single time points and therefore reflect the rates but not necessarily the extent of fatty acid formation. Moreover, those observations may not be relevant to older animals, since development of the adult pattern of intestinal transport mechanisms in the rat only begins in the third week (Batt and Schachter, 1969). Comparisons between single dose studies and studies in mature rats that had received daily doses for 90 days suggest that changes do not occur with an agent such as polydextrose (Figdor and Rennhard, 1981). Of particular relevance, studies in adult humans following repeated doses of polydextrose for 7 days yielded caloric utilization values similar to those in rats receiving single doses (Figdor and Bianchine, 1983). Studies in adult rats (or humans) presumably started with intestinal flora able to digest the bulking agents as well as was possible under these circumstances. The absence of adaptation in disposition studies is therefore not unexpected.

Registry No. Sorbitol, 50-70-4; isomalt, 64519-82-0.

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Enzyme Electrode for the Determination of Sucrose in Food Products

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A multienzyme electrode for the determination of sucrose in food and agricultural products was developed. Both the immobilization and experimental parameters were optimized. The electrode was evaluated for the determination of sucrose over analogous interferences. For sucrose, the linear dynamic range is 3.33×10^{-5} to 1.3×10^{-3} M for the initial-rate method and 3.3×10^{-5} to 1.5×10^{-3} M for the steady-state method. The electrode is very stable and gives fast response to sucrose.

Immobilized enzymes are receiving increasing attention (Guilbault, 1984). Although Clark and Lyon (1962) first introduced the concept of the "soluble" enzyme electrode, the first working electrode was reported by Updike and Hicks (1971). Today, enzyme electrodes are applied to the determination of a wide variety of sugars such as glucose (Clark and Lyon, 1962; Guilbault and Lubrano, 1973; Pfeiffer et al., 1979), lactose (Bertrand et al., 1981; Frank and Christen, 1984; Mason, 1983), galactose (Taylor et al., 1977), and maltose (Coulet and Bertrand, 1979). Techniques such as polarimetry, isotope dilution, chromatography, refractometry, and densitometry have been developed and employed for the determination of disaccharides, mainly sucrose (Schneider, 1982). These methods generally require laborious sample pretreatment

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