nm, 290 sh, 263; (NaOMe) 360 nm, 268, 240 sh. The absence of shifts with $AlCl_3$ is characteristic of flavonoids that do not contain hydroxyl groups at carbons 5, 6, and 7. A bathochromic shift with sodium methoxide is indicative of a sugar or hydroxyl at the 7 position. Hydrolysis of S_7 with H_2SO_4 gave fraction S_8: ^1H NMR (CD_3OD) δ 7.88–7.56 (2 H, m, H-2′, H-6′), 7.53–7.48 (3 H, m, H-3′, H-4′, H-5′), 6.81 (1 H, s, H-3), 6.63 (1 H, s, H-8), 3.91 (3 H, s, 5-OCH_3), 3.89 (3 H, s, 6-OCH_3) (Massicot and Marthe, 1962). The presence of a methoxyl group at C-5 is shown by proton resonance at 3.95–4.05 ppm (Anker et al., 1969) and with a shift of 0.33 ppm downfield upon the addition of trifluoroacetic acid. The mass spectrum of S_8 was as follows: MS m/e (rel intensity) 298 (9, M^+) 297 (7), 283 (100), 267 (9), 255 (10), 241 (3). The M – 1 fragment confirms the methoxy group (Kingston, 1971). Cellulose TLC (solvent = BuOH–pyridine–water, 9:5:4 v/v/v) of the acid hydrolysate after neutralization with $BaCO_3$ gave glucose. The conclusion is that S_7 is ovatin (5,6-dimethoxyflavone 7-*O*-glucoside).

DISCUSSION

The root growth inhibition of individual flavones and aglycons from *S. ovata* was no greater than 20% at 100 ppm. Chrysin had this strongest activity, reducing radish root growth 20% at 100 ppm when compared to the control.

Five of the flavonoids found in *S. ovata* have previously been observed in various species of *Scutellaria* indigenous to Europe. The aglycon of oroxylin 7-*O*-glucoside (S_1, Figure 2) was isolated and identified by Naylor and Chaplin (1890) from *Oroxylin indium* and synthesized by Rivaille and Mentzner (1965). Ovatin (S_7, Figure 3) has not been found in *Scutellaria* previously but is related to biacalein (5,6-dihydroxyflavone 7-*O*-glucoronide) found in the root of *S. biacalensis* (Bargellini, 1919) and *S. scordifolia* (Popova et al., 1976). Most of the flavones isolated from *Scutellaria* species are characterized by the absence of hydroxyl groups on the B ring, which is rare among flavonoids (Harborne, 1964).

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Received for review April 13, 1981. Revised manuscript received August 3, 1981. Accepted August 3, 1981. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Caloric Utilization and Disposition of [^{14}C]Polydextrose in the Rat

Sanford K. Figdor* and Hans H. Rennhard

Polydextrose is a tasteless, nonsweet, low caloric bulking agent formed by the random polymerization of glucose with lesser amounts of sorbitol and citric acid. It is not absorbed after oral administration, and the major portion (~60%) of polydextrose is excreted in the feces. A fraction of fed polydextrose (~30%) is fermented in the lower gut by the intestinal microflora to products such as volatile fatty acids (VFA) and CO_2; the VFA are caloric to the host, but the CO_2 is not. Metabolic studies show that polydextrose has approximately 1 cal/g, or about 25% the value of glucose. Polydextrose can serve as a total or partial replacement for sugar and as a partial replacement for fat and flour in a variety of common processed foods with accompanying caloric reduction of those foods.

The regulation of caloric intake and body weight control for most individuals is desirable but not always readily achievable within the context of modern affluent society. For the most part, caloric control is accomplished by simply limiting the intake of calories so that it is less than or equal to the energy expenditure. Some personal discomfort is commonly experienced while achieving desired goals of weight control.

An attractive approach toward the problem of weight regulation is the normal ingestion of tasteful foods which are devoid of or deficient in calories. It is unreasonable to expect that an entire palatable, nutritious, and varied diet may be assembled that is essentially free of calories. It is not unrealistic, however, to expect that a moderate portion of a daily diet may be replaced with palatable foods having reduced caloric content.

We report on a substance, polydextrose, which resists enzymatic and microbial attack and thus forms the basis for its utilization as a water-soluble, low caloric bulking agent in food products (Rennhard, 1973, 1981). Applications in a wide variety of foodstuffs show that polydextrose can serve as a total or partial bulk replacement for sugar and as a partial replacement for fat and flour. It may be incorporated into an assorted spectrum of foods such as baked products, candies, ice cream, puddings, salad

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0021-8561/81/1429-1181$01.25/0

dressing, etc. (Beereboom, 1979; Torres, 1981).

Metabolic studies in the rat, presented in this communication, and man (Figdor, 1981) have established that polydextrose has approximately 1 cal/g, or about 25% of the caloric value of glucose, and thus represents a substantial sparing of calories in whatever food is prepared from it. Since polydextrose is tasteless and is deficient in calories, it closely approximates the ideal concept of a substance that may be freely ingested yet contains a deficit of calories.

Polydextrose is prepared by the melt polymerization in vacuo of a mixture of glucose, sorbitol, and citric acid (approximate ratio of 90:10:1) (Rennhard, 1973). It is a randomly bonded polymer of glucose with sorbitol end groups and citric acid attached to the polymer by mono- and diester bonds. Because of the reactivity of the primary hydroxy group, the 1–6 bond predominates in the polymer, but degradation studies involving classical polysaccharide structural investigation techiques show that all of the other possible bonds are present. The effect of the random bonding and occassional diester cross-linking is a polymer that is more resistant to acid or enzyme hydrolysis than a corresponding regular polymer such as starch (Rennhard, 1973, 1981).

Polydextrose is not absorbed after oral administration, and the major portion is excreted unchanged in the feces. A fraction of fed polydextrose is fermented in the lower gut by the intestinal microflora to products such as volatile fatty acids and CO_2. Some of the CO_2 so formed is absorbed and is subsequently eliminated; it is not caloric. The volatile fatty acids are absorbed by the mammalian host and are a source of calories; they are also eventually eliminated, for the most part, as CO_2. $^{14}CO_2$ recovery experiments performed in rats and man with [^{14}C]polydextrose indicate that approximately $^1/_3$ of an orally administered dose is converted to $^{14}CO_2$.

We describe below experiments conducted with carbon-14-labeled polydextrose in the rat which characterize the caloric utilizaton and disposition of polydextrose.

EXPERIMENTAL SECTION

Preparation of [^{14}C]Polydextrose. A mixture of 90 parts of [^{14}C]-D-glucose, 10 parts of [^{14}C]sorbitol (both uniformly labeled), and 1 part of nonlabeled citric acid was heated in vacuo at 190 °C for 20 min or at 160 °C for 3 h. The molecular weight profiles of both mixtures are virtually identical. Minor byproducts that are present in the reaction mixture consist of unreacted glucose, sorbitol, and citric acid, as well as 1,6-anhydroglucose and hydroxymethylfurfural. The latter two substances occur widely in foods since they are formed whenever sugar is heated (caramel formation) or starch is acid degraded (corn syrup). Since these commonly used food components were not of interest, they were removed from the reaction mixture by Sephadex G-15 gel filtration. The resulting radioactive product is essentially purified polymer free of monomers. In a typical procedure the molecular weight profile of [^{14}C]polydextrose used in these experiments is

molecular weight range	%
250–5 000	88.7
5 000–10 000	10.0
10 000–16 000	1.2
16 000–18 000	0.1

Rennhard (1981) has presented a more complete discussion of polydextrose synthesis and properties.

Animal Studies. Rats that weighed between 125 and 200 g each received [^{14}C]polydextrose in water solution by gavage (1 mL) or by intravenous administration into the tail vein (0.5 mL). Some rats were placed in plastic metabolism cages (Microchemical Specialties) that permitted the separate and total collection of urine and feces. Collections were generally carried out for 3 days. In those experiments where $^{14}CO_2$ was collected, rats were placed immediately after dosing into an Aerospace Industries rat restrainer and metabolism cage. $^{14}CO_2$ was collected by pulling CO_2-free, dry air through the metabolism cage and subsequently through a column of 200 mL of 10% sodium hydroxide. The CO_2 traps were changed every hour for the first 13 h, and an aliquot of the sodium hydroxide solution was assayed for radioactivity as described below. In some experiments the $^{14}CO_2$ collection was extended to 24 h. The Aerospace Industries metabolism cage permits the separate and total collection of urine and feces during the course of the $^{14}CO_2$ collection. When the $^{14}CO_2$ collection portion of an experiment was completed, the rat was transferred to a conventional metabolism cage, and urine and feces were separately collected for the remainder of the 3-day period.

Intravenous Administration. Three rats weighing 200 g each received an intravenous dose of [^{14}C]polydextrose at 25 mg/kg (13.8 μCi, rat no 32) or 50 mg/kg (13.4 μCi, rat no. 34, and 23.3 μCi, rat no. 35). Collection of $^{14}CO_2$ was carried out at hourly intervals for 13 h and as a single collection for the interval 13–24 h.

Oral Administration. Rats weighing 200 g each received a single dose of 55 mg/kg [^{14}C]polydextrose (12.6 μCi) by gavage. Collection of $^{14}CO_2$ was conducted at hourly intervals for 13 h. Rat no 15 was retested 1 week after the first experiment.

Stressed Rats. Groups of rats had free access to normal rat chow containing nonlabeled polydextrose at a concentration equivalent to 1 or 10 g (kg of body weight)$^{-1}$ day^{-1} for 90 days. A group of rats eating normal food served as a control. All rats used in this tracer study were males.

At the end of 90 days two rats were selected from each of the three groups and received 1 mL of an aqueous solution containing 14.7 mg of [^{14}C]polydextrose (total radioactivity = 36.7 μCi/dose). The labeled dose was approximately 30 mg/kg, depending upon individual rat weights. The rats weighed between 460 and 650 g each. Immediately after receiving the tracer dose each rat was placed in a metabolism chamber designed to collect the exhaled $^{14}CO_2$. Expired breath was collected at hourly intervals for 13 h, and urine and feces were separately collected at 24-h intervals for 3 days.

Recycled [^{14}C]Polydextrose in Rats. Rats weighing approximately 200 g each received a water solution of [^{14}C]polydextrose by gavage. The 0–24-h fecal collection, containing approximately 50% of the administered radioactivity, was homogenized in water and extracted with several small portions of water. The combined aqueous extracts were centrifuged and the supernatant solution was reduced in volume by lyophilization. Purification of the concentrate was achieved by gel filtration over Sephadex G-15. Appropriate Sephadex fractions were combined, reduced in volume to 1–2 mL and fed to rats by gavage. Rat no. 11 received 3.2 μCi of recovered [^{14}C]polydextrose, rat no. 16 received 3.8 μCi, and rat no. 22 received 2.5 μCi.

Oral Absorption of Unchanged Polydextrose by the Rat. A rat weighing 202 g was stressed for 3 days by oral intubation with a daily dose of nonlabeled polydextrose at 5 g kg^{-1} day^{-1}. On the fourth day the rat received 5 g/kg [^{14}C]polydextrose containing 23 μCi of radioactivity and was immediately placed in a metabolism cage designed to separate urine and feces. For comparison purposes a rat

weighing 225 g received 1 g kg^{-1} day^{-1} of nonlabeled D-glucose by oral intubation for 3 days. On the fourth day the rat was given 1 g/kg [^{14}C]-D-glucose (uniformly labeled) at a total radioactive dose of 20 μCi.

Since most of the administered polydextrose is excreted unchanged with feces, contact of urine with feces could leach polydextrose into the urine. So that this complication is avoided, the rat was restrained and positioned to rigorously prevent possible mixing of urine and feces.

Polydextrose was isolated from urine by gel filtration with Sephadex G-10, G-15, or G-50 using nonbuffered water solutions. For identification the polymer was hydrolyzed to α-glucose, β-glucose, and sorbitol. These three components of polydextrose were analyzed by gas–liquid chromatography and mass spectrometry.

Polydextrose and urine-containing polydextrose samples were hydrolyzed to a mixture of α-glucose, β-glucose, and sorbitol by refluxing in 1 N HCl for 3 h. The samples were neutralized with silver carbonate, centrifuged, and lyophilized. The dry residue was treated with excess *N*-(trimethylsilyl)imidazole in pyridine and assayed by gas–liquid chromatography with appropriate reference standards.

GLC was carried out in a Hewlett-Packard Model 402 using glass columns. $^1/_4$ in × 6 ft and containing 3% OV-1 on Chromosrob W. The column temperature was 170 °C, the inlet temperature was 230 °C., and the detector temperature was at 240 °C. A hydrogen flame ionization detector was employed. Carrier gas was helium at 60 mL/min.

Mass spectra were obtained on the LKB gas chromatograph–mass spectrometer, Type 9000. The effluent from the gas chromatograph was split between a conventional detector and the mass spectrometer. It was therefore possible to monitor the GLC by conventional detector methods and simultaneously obtain mass spectra of appropriate fractions.

Volatile Fatty Acids (VFA) in the Intestinal Flora of the Rat after [^{14}C]Polydextrose. Two experiments are described. (1) The first is administration of polydextrose by gavage to rats and analysis of feces. Rats weighing 150–200 g received 28 μCi of [^{14}C]polydextrose as an aqueous solution and feces collected during the interval 0–24 h after the label dose were used for the assays. (2) The second is investigation of rat cecal contents. A rat dosed as above was killed 5 h after receiving the [^{14}C]polydextrose. The cecum was removed, and the contents were suspended in water and subsequently processed as described below for feces.

Feces, or cecal contents, were homogenized in water and extracted with many small portions of water. The combined extracts were adjusted to pH 8.1 with 1 N sodium hydroxide and lyophilized. The residue was made acid with sulfuric acid and steam distilled. The distillate was adjusted to pH 8.5 and lyophilized, and the residue was treated with dichloroacetic acid. After centrifugation, the supernatant radioactive solution was analyzed by GLC. GLC conditions were as follows: instrument, Perkin-Elmer gas chromatograph, Model 881, with an FID and radioactivity monitor; column, $^1/_8$ in. × 6 ft (glass), packed with 3.75 g of 10.5% ethylene glycol adipate plus 1.75% phosphoric acid and Anakrom ABS, 110–120 mesh; column temperature, 90 °C. Nonradioactive acid standards were used to establish the appropriate retention times.

Radiochemical Methods. Samples were assayed in a Nuclear Chicago Mark I liquid scintillation spectrometer. Quench correction was performed by the method of internal standardization using [^{14}C]toluene.

Table I. Intravenous Administration of [^{14}C]Polydextrose to Rats

	% of administered dose			
	rat no. 32	rat no. 34	rat no. 35	mean ± SD
CO_2[a]	1.07	1.37	1.05	1.16 ± 0.18
caloric utilization[b]	1.78	2.28	1.75	1.93 ± 0.30
urine: 0-3 h	79.3	103.0	88.3	90.3 ± 12.2
3-24 h		0.3		
24-48 h	0.3	0.3	0.2	0.3
48-72 h	0.2	0.2	0.2	0.2
total urine	79.7	103.8	88.7	90.7 ± 12.2
feces: 0-24 h	7.9	0.9	0.2	3.0 ± 4.3
24-48 h	2.3	0.9	0.1	1.1 ± 1.1
48-72 h	0.3	0.4	0.2	0.3 ± 0.1
total feces	10.5	2.2	0.5	4.4 ± 5.4
total urine feces plus caloric utilization	92.0	108.3	91.0	97.1 ± 9.7

[a] $^{14}CO_2$ collected for 24 h. [b] See Caloric Utilization for a discussion of the use of $^{14}CO_2$ exhalation as an indicator of caloric utilization.

$^{14}CO_2$ was assayed by pulling a stream of dry CO_2-free air through the metabolism cage and then through a trap of 200 mL of a 10% sodium hydroxide solution. The sodium hydroxide trap was changed every hour for the first 13 h. Only one trap was used for the interval of 13–24 h. Aliquots of the sodium hydroxide solution were assayed in a 30/70 scintillator (see below).

(1) *Urine.* Aliquots of urine or diluted urine were assayed in 30/70 scintillator with added Triton X.

(2) *Feces.* Collections were homogenized in a Waring Blendor and lyophilized. Aliquots of the resulting powder were combusted in an Oxymat (Intertechnique) combustion unit with appropriate reference standards. The efficiency of combustion was generally around 95%.

The 30/70 scintillator was prepared with Omnifluor (New England Nuclear) and consisted of 0.0327% PPO and 0.0067% bis-MSB in a solution of 30% absolute ethanol and 70% toluene. The Triton X scintillator contained Triton X added to Omnifluor dissolved in toluene.

Caloric Utilization. The caloric utilization of polydextrose is calculated from the quantity of $^{14}CO_2$ obtained from rats after labeled polydextrose administration. Experiments in these laboratories have shown that the administration to rats of uniformly labeled [^{14}C]glucose, a substance which is rapidly absorbed and utilized, results in the exhalation of approximately 60% of the radioactivity as $^{14}CO_2$ within 24 h. Maximum exhalation of $^{14}CO_2$ occurs at 1 or 2 h after the dose. Thus, although all of the glucose is utilized by the rat, only a portion is exhaled as $^{14}CO_2$. A survey of the literature shows that a total of 60% of an available carbon source, such as [^{14}C]acetate, is exhaled by mammals as $^{14}CO_2$ in 24 h (Shreeve et al., 1959; Hellman et al., 1951; S. K. Figdor and H. H. Rennhard, unpublished experiments; following the administration of an aqueous solution of uniformly labeled [^{14}C]-D-glucose or [1-^{14}C]acetate by gavage to rats, 62.3 and 55.4%, respectively, of the radioactivity was recovered as $^{14}CO_2$ within 24 h). Consequently, to estimate total caloric utilization of polydextrose, the actually recovered $^{14}CO_2$ is corrected by the catabolic conversion factor (acetate → CO_2) of 0.6.

RESULTS AND DISCUSSION

Intravenous Administration of Polydextrose to Rats. The recovery of radioactivity from rats is summarized in Table I. An average of 1.16% of the administered dose was recovered as $^{14}CO_2$, indicating that only 1.93% of dose was available to the rat and used as calories (see

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

	% of administered dose				
	rat no. 15	rat no. 15[a]	rat no. 16	rat no. 17	mean ± SD
$^{14}CO_2$[b]	20.6	24.9	17.3	20.6	20.9 ± 3.1
caloric utilization	34.6	41.5	28.9	34.4	34.8 ± 5.2
urine: 0-24 h	1.30	1.78	1.34	1.48	1.5 ± 0.2
24-48 h	0.11	0.12	0.31	0.32	0.2 ± 0.1
48-72 h		0.08	0.07	0.18	0.1 ± 0.06
total urine: 0-72 h	1.41	1.98	1.72	1.98	1.8 ± 0.3
feces: 0-24 h	65.4	51.8	48.0	35.9	50.2 ± 12.2
24-48 h	1.3	1.9	18.9	13.7	9.0 ± 8.8
48-72 h	0.3	0.4	1.4	2.1	1.1 ± 0.9
total feces: 0-72 h	67.0	54.1	68.3	51.7	60.3 ± 8.6
total urine plus feces plus caloric utilization	102.8	97.6	98.9	88.1	96.9 ± 6.2

[a] Rat no. 15 received a second dose of [^{14}C]polydextrose 1 week after the first dose. [b] $^{14}CO_2$ was collected for 13 h.

Caloric Utilization for a discussion of the use of $^{14}CO_2$ exhalation as an indicator of caloric utilization). The major portion of administered radioactivity is excreted in urine. In one experiment, with rat no. 34, essentially all of the urinary radioactivity was recovered within 3 h of dosing. Recovery of radioactivity in feces was variable and extended from 0.5% (rat no. 35) to 10.5% (rat no. 32). The total recovery of radioactivity by all routes was 97.1%.

Comprehensive studies with polymeric Dextran plasma expanders have shown that renal clearance rates are a function of the molecular weights of the polymers. Dextran with molecular weights up to 16 000 is cleared with creatinine, a universal standard for renal clearance studies. Molecules of this size are eliminated rapidly by glomerular filtration with an intravascular half-life of about 15 min (Arturson and Wallenius, 1964; Arturson et al., 1971). Larger Dextran molecules of molecular weight approximating 20 000 are cleared less rapidly, at about 50% the rate of creatinine. Analysis of molecular weight profiles of polydextrose used in these experiments indicates that 99% of the material is less than molecular weight 15 000 and that nearly 90% is less than 5000. Therefore it is to be expected that after IV administration, polydextrose will be rapidly and completely eliminated with urine. Because practically all polydextrose recovered from urine is excreted within 3 h, it can be concluded that the half-life is less than 30 min and that the relative volume of distribution is less than total body water. polydextrose appears to be confined to the extracellular space.

The recovery of $^{14}CO_2$ after intravenous [^{14}C]polydextrose administration is approximately 1% of dose. Thus the rate of polydextrose degradation by mammalian enzymes is slow or nonexistent when compared to its rate of elimination.

Oral Administration of Polydextrose to Rats. The recovery of radioactivity after the oral administration of [^{14}C]polydextrose is shown in Table II. Approximately 20% of the administrated radioactivity is recovered as $^{14}CO_2$, less than 2% in urine, and the remainder in feces. Practically all of the radioactivity that is recovered in urine and feces is obtained within the first 24 h after dosing.

Figure 1, curve A, shows the time course of $^{14}CO_2$ exhalation after [^{14}C]polydextrose administration over the 13-h collection period. Maximum excretion rates of $^{14}CO_2$ were found 6–8 h after ingestion of [^{14}C]polydextrose, indicating that the product was not absorbed from the upper gastrointestinal tract but had reached the lower gut, where the microflora converted polydextrose to small molecules, which then were absorbed by the rat.

Although the major fraction of $^{14}CO_2$ was recovered during the 6–10-h interval after polydextrose administration, minor amounts of $^{14}CO_2$ were obtained during the second and third hour (Figure 1). The recovery of $^{14}CO_2$

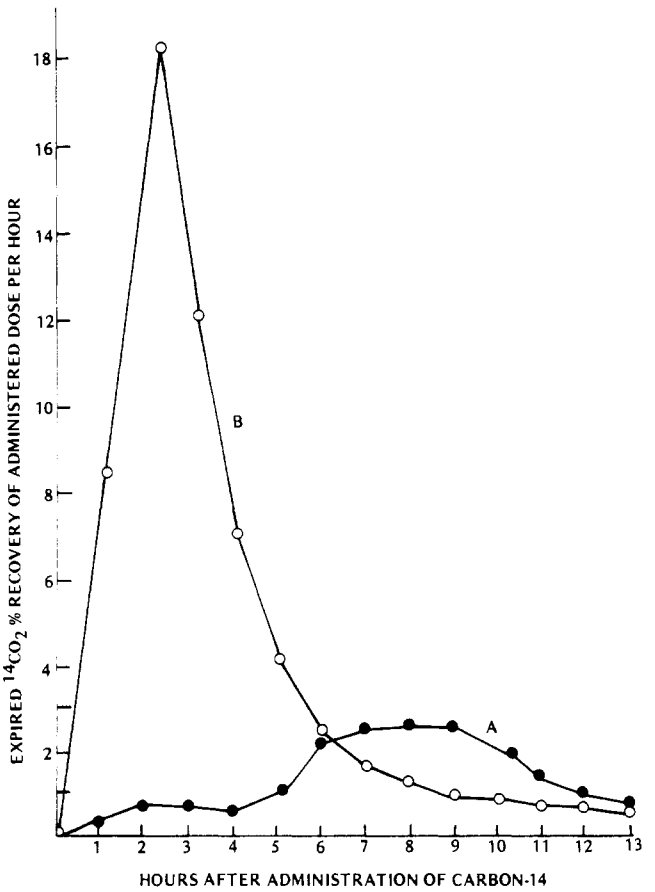

Figure 1. Recovery of expired $^{14}CO_2$ from the rat after a single oral dose of (A) [^{14}C]polydextrose or (B) [^{14}C]glucose.

during this early time interval is indicative of facile absorption from the upper gastrointestinal tract followed by rapid metabolism to CO_2 (see discussion of [^{14}C]glucose below). The [^{14}C]polydextrose employed in these studies was purified by gel filtration to remove any unreacted monomers. It is possible, however, that some small amount of labeled monomer remained with the polymer and was responsible for the early $^{14}CO_2$ peak seen in Figure 1. Alternatively, since polydextrose is a randomly bonded polymer, some of the saccharide bonds may be susceptible to acid or enzyme hydrolysis in the stomach. This in turn may result in the release of a small quantity of monosaccharides which are then rapidly absorbed and converted to $^{14}CO_2$. The available data do not permit a choice between these two hypotheses nor exclude the possibility that both occurred.

For comparison, Figure 1, curve B, shows the time course of $^{14}CO_2$ exhalation after [^{14}C]glucose administration, a

saccharide which is absorbed rapidly from the upper gastrointestinal tract. The maximum exhalation of $^{14}CO_2$ occurs as a sharp spike 2 h after administration and indicates rapid absorption and metabolism.

As shown in Table II, approximately 20% of dose recovered was $^{14}CO_2$ after [^{14}C]polydextrose administration, indicating a maximum caloric utilization of approximately 35% of dose. For the reasons cited below, the true caloric utilization of polydextrose by the rat is less than 35%. The administration to rats of polysaccharides that are not absorbed may result in the recovery of $^{14}CO_2$ in the breath, but the pathways and mechanism differ from those for glucose. Oligosaccharides are not absorbed directly but pass on to the lower gut where they serve as a substrate for fermentation by the intestinal microflora (Steggerda, 1968). Two product types are generated by this fermentation process: small organic molecules, including fatty acids and alcohols, and gasses such as CO_2, each with a different potential as a source of calories to the mammalian host. The small chain fatty acids (and alcohols) that are elaborated by fermentation are absorbed by the host and metabolized to CO_2 which eventually appears in the breath. Those small organic compounds provide calories to the host. The CO_2 formed directly from the fermentation is lost as flatus, as well as absorption into the blood stream from which it is removed and eventually exhaled. However, this CO_2 does not supply calories to the host. Thus, after the oral administration of the polysaccharide [^{14}C]-polydextrose, the $^{14}CO_2$ collected is obtained from two sources; mammalian enzyme-degraded fatty acids which were derived from the bacterial fermentation of polydextrose (caloric) and $^{14}CO_2$ obtained directly from the fermentation in the gut (noncaloric). On the basis of studies with [^{14}C]polydextrose in man in which only exhaled $^{14}CO_2$ was collected, it can be estimated that gut fermentation derived $^{14}CO_2$ in the rat constitutes at least $^1/_3$ of the total collected $^{14}CO_2$ (Figdor and Bianchine, 1981). Since it is not possible to distinguish $^{14}CO_2$ derived from fatty acids from $^{14}CO_2$ produced by microorganisms, caloric utilization estimates which are based upon recovered $^{14}CO_2$ are high estimates, due to the inclusion of noncaloric $^{14}CO_2$ obtained directly via fermentation (see also Summary).

The low urinary recovery of radioactivity confirms that very little polydextrose is absorbed as such. Most of the radioactivity present in urine represents normal waste products (i.e., urea) labeled by incorporation of carbon-14 by the usual metabolic processes. As shown in the intravenous study, any polydextrose that would have passed into the general circulation is immediately excreted by the kidneys. Consequently, it can be concluded that the animals absorbed only compounds which are almost without exception always found in the gut following ingestion of any normal diet. Systemic exposure to polydextrose is minute and toxicologically insignificant as shown below.

Formation of Radioactive Volatile Fatty Acids from [^{14}C]Polydextrose by the Intestinal Flora of the Rat. It has been shown that the degradation and utilization of cellulose by the rat is qualitatively identical with that in ruminants, namely, fermenation by intestinal microorganisms to a mixture of volatile fatty acids (Albanese, 1963). The two studies discussed below were carried out to verify that polydextrose is also subject to bacterial fermentation and to identify radioactive VFA in rat feces after [^{14}C]polydextrose administration.

Radioactive VFA in Rat Feces After Feeding [^{14}C]Polydextrose. Rat feces collected during the 24-h interval after the oral administration of [^{14}C]polydextrose were extracted and steam distilled as described. Simultaneous

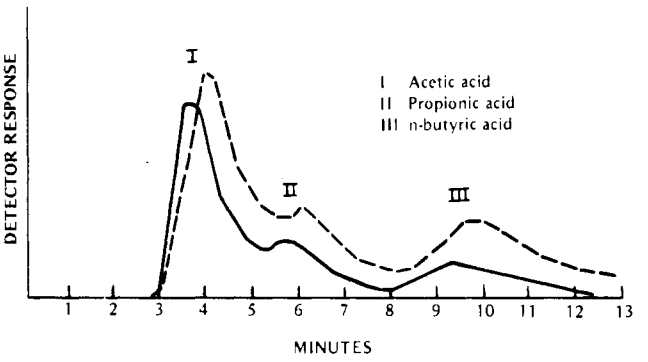

Figure 2. Gas chromatography of volatile fatty acids isolated from rat feces by steam distillation after oral administration of [^{14}C]polydextrose. (—) FID response; (---) radioactivity monitor response (delay ca. 40 s).

gas–liquid chromatography (GLC) and radioactivity monitoring demonstrated the presence of radioactive acetic, propionic, and butyric acids in the relative abundance shown in Figure 2. The steam distillate contained 2.5% of the excreted fecal radioactivity.

Rat Radioactive Cecum Contents. Five hours after receiving an oral dose of [^{14}C]polydextrose, 58% of the administered radioactivity was recovered from the rat cecal contents. Following the described procedure, the final steam distillate contained 13% of the administered label. Analysis of the steam distillate by GLC and radiomonitoring resulted in a pattern similar to that shown in Figure 2, and the relative abundance of VFA was acetic acid > butyric acid > propionic acid.

The results obtained from intravenous and feeding experiments show that [^{14}C]polydextrose is not metabolized after iv administration but is partially metabolized after oral administration, thus implicating the intestinal microflora. The delayed time course of $^{14}CO_2$ exhalation supports the proposition that polydextrose is not absorbed but is fermented in the lower gut. The experiments described show the presence of radioactive VFA in rat feces after [^{14}C]polydextrose administration. In addition, rat cecum contents contain a significant portion of the administered dose (13%) as VFA 5 h after ingestion of [^{14}C]polydextrose. Thus, these experiments confirm that polydextrose is fermented by the microflora in the lower intestinal tract, resulting in the production of volatile fatty acids.

Disposition of [^{14}C]Polydextrose in Rats That Received Large Doses of Polydextrose for 90 Days (Stressed Rats). The purpose of this experiment was to determine if rats that have been fed large daily doses of polydextrose for 90 days adapt to this material or show altered caloric utilization or metabolism of polydextrose.

The recovery of exhaled $^{14}CO_2$ is shown in Table III from rats that received a standard oral dose of [^{14}C]polydextrose after 90 days feeding with nonlabeled polydextrose at 1 or 10 g kg^{-1} day^{-1}. The average $^{14}CO_2$ collection expressed as percent of the administered dose was 18.7% for the controls, 19.6% for the 1 g/kg group, and 18.5% for the 10 g/kg group. The time course of exhaled $^{14}CO_2$ from the control (untreated) rats was not significantly different from that observed in the polydextrose-fed rats. All groups exhibited the same pattern of a broad maximum exhalation of $^{14}CO_2$ over the interval of approximately 6–10 h (see Figure 1). Urinary recovery of label was also identical for the three groups and averaged 1–2% of the administered dose. The fecal recovery was virtually the same for the control and 1 g/kg rats, averaging approximately 50% of dose (Table IV). Those rats receiving 10 g/kg polydextrose demonstrated somewhat lowered fecal recovery;

Table III. Recovery of $^{14}CO_2$ following a Single Dose of [^{14}C]Polydextrose to Stressed Rats

	% of administered radioact. recovered as $^{14}CO_2$					
	control (nontreated)		1 g/kg polydextrose for 90 days		10 g/kg polydextrose for 90 days	
time, h	rat no. 33774	rat no. 33775	rat no. 33804	rat no. 33805	rat no. 33784	rat no. 33785
1	0.58	0.48	0.37	0.50	0.33	0.49
2	1.04	0.93	0.76	0.91	0.66	0.91
3	0.84	0.74	0.77	0.65	0.65	0.71
4	0.73	0.80	0.66	0.92	0.54	0.69
5	1.01	1.29	1.11	2.42	0.92	0.97
6	1.74	1.66	2.28	3.22	2.20	2.19
7	2.04	2.19	2.61	2.33	2.31	2.99
8	2.02	2.59	2.66	2.16	2.04	2.65
9	1.94	2.58	2.62	1.89	1.72	2.60
10	1.81	2.16	1.96	1.80	1.07	2.41
11	1.22	2.26	1.44	1.36	1.25	2.39
12	1.06	1.57	0.96	1.13	0.81	1.57
13	0.80	1.31	0.70	0.95	0.75	1.15
total $^{14}CO_2$	16.83	20.56	18.90	20.27	15.25	21.72
$^{14}CO_2$ collected, av of both rats from each group	18.69		19.58		18.48	
caloric utilization	28.08	34.26	31.50	33.78	25.42	36.20
caloric utilization, av of both rats from each group	31.2		32.6		30.8	

Table IV. Urinary and Fecal Excretion of Radioactivity from Stressed Rats after [^{14}C]Polydextrose Administration

	% of administered dose recovered					
	control (nontreated)		1 g/kg polydextrose		10 g/kg polydextrose	
	rat no. 33774	rat no. 33775	rat no. 33804	rat no. 33805	rat no. 33784	rat no. 33785
urine: 0-24 h	1.36	1.56	1.65	0.99	2.98	1.01[a]
24-48 h	0.37	0.15	0.06	0.12	0.25	0.34
48-72 h	0.08	0.06	0.05	0.05	0.09	0.11
total urine: 0-72 h	1.81	1.77	1.76	1.16	3.32	1.46
average of both rats from each group	1.8		1.5		2.4	
feces: 0-24 h	50.63	43.23	55.62	41.31	25.29	37.05
24-48 h	2.55	10.07	2.18	2.19	12.62	6.78
48-72 h	1.09	1.62	0.28	0.39	0.95	0.11
total feces: 0-72 h	54.27	54.92	58.08	43.89	38.86	43.94
total urine and feces: 0-72 h	56.08	56.69	59.84	45.05	42.18	45.40
average of both rats from each group	56.4		52.4		43.8	

[a] Some urine was lost during this collection period.

however, this was due mainly to problems associated with collection (Table IV). During the $^{14}CO_2$ collection, rats are kept in a special restraining holder which is designed to separate and collect urine and feces. However, rats on a high dose of polydextrose (10 g kg^{-1} day^{-1}) excrete very soft feces which do not fall into the receptacle but tend to "smear" over the restraining cage as well as the rat. Every effort is made to collect these excreta, but it is virtually impossible to recover this material from the rat fur.

Thus rats stressed by feeding polydextrose at 1 or 10 g kg^{-1} day^{-1} for 90 days metabolize a test done of [^{14}C]polydextrose quantitatively and qualitatively the same as control rats which were not fed polydextrose. No induction of microbial metabolism was observed.

Recycled [^{14}C]Polydextrose in the Rat. Rats were fed [^{14}C]polydextrose recovered from the feces of rats previously fed [^{14}C]polydextrose to estimate the extent of utilization during the second passage through a rat.

A summary of the results obtained from feeding three rats recycled [^{14}C]polydextrose is shown in Table V. Less than 6% of the dose is recovered as $^{14}CO_2$ which indicates a caloric utilization of less than 10%, or less than $^1/_3$ of that obtained from "new" polydextrose. Approximately 1.4% of the administered radioactivity was eliminated in urine, and the major portion, approximately 90%, was recovered in feces. A comparison of the results obtained

Table V. Recycle of [^{14}C]Polydextrose in the Rat

	% of administered dose			
	rat no. 11	rat no. 16	rat no. 22	mean ± SD
$^{14}CO_2$	5.2	7.4	4.4	5.7 ± 1.6
caloric utilization	8.6	12.3	7.3	9.4 ± 2.6
urine: 0-24 h	1.28	1.05	1.43	1.25 ± 0.19
24-48 h	0.14	0.10	0.07	0.10 ± 0.04
48-72 h	0.07	0.01	0.02	0.03 ± 0.03
total urine: 0-72 h	1.49	1.16	1.52	1.38 ± 0.20
feces: 0-24	79.2	73.5	82.2	78.3 ± 4.4
24-48 h	13.0	11.3	7.9	10.7 ± 2.6
48-72 h	0.9	0.6	0.2	0.6 ± 0.4
total feces: 0-72 h	93.1	85.4	90.3	89.6 ± 3.9
total urine plus feces plus caloric utilization	103.2	98.9	99.1	100.4 ± 2.4

in "new" and "once metabolized" [^{14}C]polydextrose is listed in Table VI.

As shown in preceding experiments, polydextrose is not affected by mammalian enzymes but is fermented by the intestinal flora of the lower gut. On the basis of the results of these studies, the first passage of polydextrose through the rat intestine removes by fermentation that fraction of polydextrose molecules that serves as a substrate for bacterial enzymes. Subsequent passage of this metabolized

Table VI. Disposition of [^{14}C]Polydextrose, New and Recycled

	% of administered dose	
	new[a,b]	recycled[c]
$^{14}CO_2$	20.9	5.7
caloric utilization	34.8	9.4
urine: 0-24 h	1.5	1.3
24-48 h	0.2	0.1
48-72 h	0.1	0.0
total urine: 0-72 h	1.8	1.4
feces: 0-24 h	50.2	78.3
24-48 h	9.0	10.7
48-72 h	1.1	0.6
total feces: 0-72 h	60.3	89.6
total urine plus feces plus caloric utilization	96.9	100.4

[a] Data from Table II. [b] Average of four rats. [c] Average of three rats.

polydextrose through the rat intestine results in substantially reduced utilization as shown by the lowered recovery of $^{14}CO_2$. Once metabolized [^{14}C]polydextrose produces only $^1/_4$ to $^1/_3$ the $^{14}CO_2$ obtained with new polydextrose. As anticipated, there is a concomitant slight decrease in urinary radioactivity and a substantial increase in fecal radioactivity. It can be concluded that polydextrose contains a major fraction of polymeric material that is resistant to bacterial degradation.

The implication of these findings is that a finite upper limit exists to the caloric utilization of polydextrose. In the event that the passage of polydextrose through an organism is delayed, fermentation will not proceed indefinitely but will abate once those molecules that were available as substrates for the microflora are consumed. The remaining molecules are mostly resistant to further bacterial action.

Absorption (Persorption) of Intact Polydextrose by the Rat. After the oral administration of [^{14}C]polydextrose to the rat, approximately 1% of the administered radioactivity is excreted in the 0–24-h urine (Table II). It is expected that some of this urinary radioactivity will consist of normal radioactive endogenous end product metabolites derived from the absorption of radioactive fatty acids. However, some of the urinary radioactivity may also consist of [^{14}C]polydextrose since some macromolecular molecules have been shown to cross the epithelial barrier in small quantities. This has been demonstrated with ferritin (Bockman and Winborn, 1965, 1966), horseradish peroxidase, M_r 40000 (Warshaw et al., 1971), starch granules (Volkheimer et al., 1968), and partially degraded carrageenan, M_r 20000 (Abraham et al., 1972). It is therefore not unreasonable to expect that some orally administered [^{14}C]polydextrose may be absorbed and subsequently excreted in urine. Studies that were conducted to estimate the amount of polydextrose that was absorbed and subsequently excreted in the urine show that in the rat less than half of urinary radioactivity is due to polydextrose. Thus, in the rat, in terms of fed polydextrose, the percent of the administered dose of polydextrose absorbed and excreted in urine is 0.24%.

The 0–24-h urine collection obtained from the rat after [^{14}C]polydextrose (0.6% of administered radioactivity) and after [^{14}C]glucose (1.9% of administered radioactivity) was examined by gel filtration. The eluted patterns of radioactivity are shown in Figure 3 (polydextrose) and Figure 4 (glucose). Peaks no. 2 and 3 seem to be normal metabolic end products since they appear in urine after glucose administration as well as after polydextrose. Peak no. 3 was found to have the same retention time as authentic [^{14}C]urea.

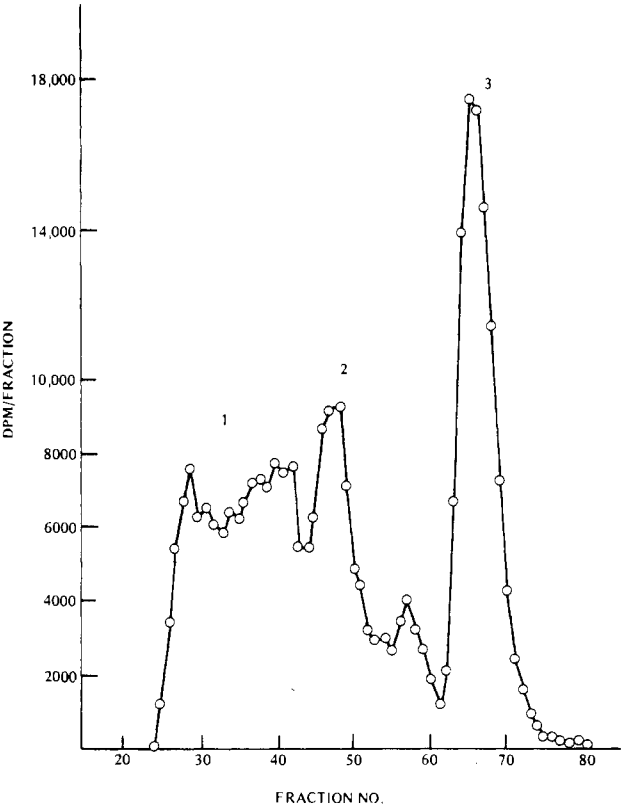

Figure 3. Elution pattern of radioactivity found in 0–24-h rat urine after oral administration of [^{14}C]polydextrose. Gel filtration on Sephadex G-15.

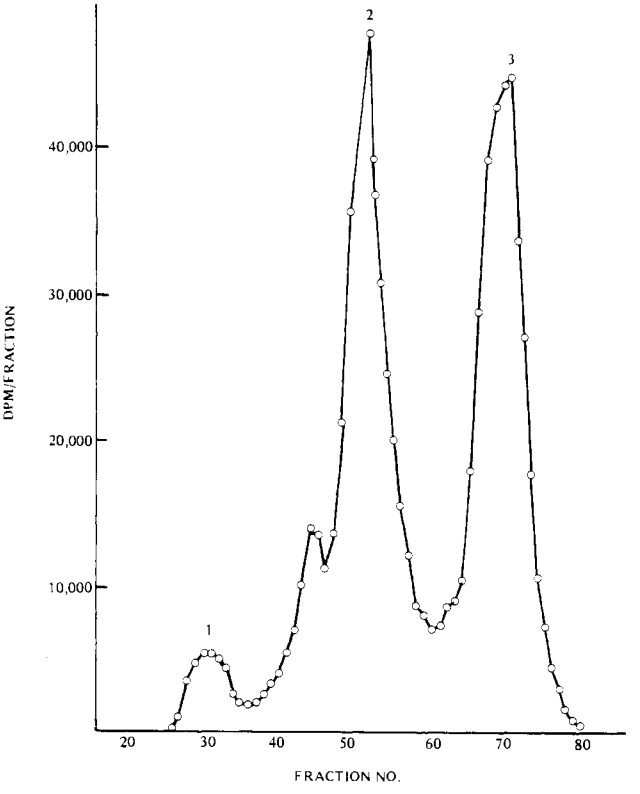

Figure 4. Elution pattern of radioactivity found in 0–24-h rat urine after oral administration of [^{14}C]-D-glucose. Gel filtration on Sephadex G-15.

The patterns of urinary radioactivity resulting from ingestion of labeled glucose or polydextrose differ mainly in the area immediately following the void volume, (peak no. 1). The polydextrose tracing (Figure 3) shows that relatively more radioactivity was obtained in this area than

from the glucose-treated rat (Figure 4). Gel filtration of authentic [^{14}C]polydextrose has shown that polydextrose is eluted from Sephadex G-15 with the void volume and thus corresponds to the radioactivity seen at peak no. 1 in Figure 3 (polydextrose).

The combined fraction no. 24–44 of Figure 3 was hydrolyzed and subjected to GLC and compared with an authentic sample of hydrolyzed polydextrose. The results showed that the pattern of α-glucose, sorbitol, and β-glucose obtained from authentic polydextrose was identical with that obtained from the rat urine sample. So that the identity of the GLC sorbitol peak was confirmed, it was analyzed by mass spectrometry and shown to be identical with that obtained from an authentic sample of sorbitol.

The radioactivity present in the combined fraction no. 24–44 of Figure 3 is approximately 41% of the total radioactivity eluted from the column. However, not all of the radioactivity is due to polydextrose, since this fraction also contains radioactivity which is derived from the normal endogenous end product metabolism of radioactive volatile fatty acids and other small molecules that are generated from the fermentation of polydextrose in the lower intestine. Therefore, results cited for the persorption of polydextrose are maximum possible amounts as some of the urinary radioactivity is not polydextrose related. Since the total urinary radioactivity recovered from this rat after [^{14}C]polydextrose administration was 0.6% of dose, a *maximum* of 0.24% of dose could have been polydextrose-related material.

In order to obtain additional information regarding the molecular weight of the absorbed polydextrose, the combined peak no. 1 fractions (Figure 3) were compared with inulin (M_r 5000) by gel filtration on Sephadex G-50. Since Sephadex G-50 will exclude substances of molecular weight greater than approximately 5000, it was anticipated that a substance with molecular weight 5000 (for example, inulin) should be excluded from the column and appear in the void volume. Substances with molecular weight less than 5000 are expected to be retained on the column and appear in later fractions collected past the void volume. Inulin was indeed eluted at the void volume, whereas the combined polydextrose fractions were absorbed on the Sephadex G-50 and subsequently eluted in later fractions. Thus, by comparison on Sephadex G-50, the polydextrose fraction is eluted behind inulin and assumed to be of lower molecular weight than inulin.

SUMMARY

The disposition by mammals of polydextrose is predictable from the structure and size of this polymer. Because the product is a randomly linked polysaccharide, it is expected to resist the attack of substrate-specific mammalian enzymes. However, the possibility that polydextrose is digested by some of the many bacterial enzymes available in the gut could not be excluded. As a polymeric carbohydrate, this food constituent is not anticipated to be absorbed and should not penetrate cell walls; yet, because the molecular weight is 1 or 2 orders of magnitude below that of the dextrans used as plasma expanders, the renal clearance of polydextrose is expected to be very rapid, and the molecules should not be retained by the reticular endothelial system.

Following the intravenous administration of [^{14}C]polydextrose, rats exhale very little $^{14}CO_2$ (approximately 1% of dose), indicating that these animals do not directly metabolize the product to any significant extent. The radioactivity is rapidly cleared by the kidneys. The mechanism of clearance is presumed to be glomerular filtration since approximately 90% of the polydextrose has a molecular weight below that of inulin (~5000), the polysaacharide employed frequently in renal clearance studies. More than 99% of label recovered with urine is excreted within 3 h after the injection of polydextrose. This rate of elimination suggests a half-life of less than 30 min and a distribution of radioactivity into extracellular space only.

After oral administration of [^{14}C]polydextrose to rats, practically negligible amounts (1–2% of dose) are found in urine. Some of this radioactivity represents unchanged polymer, indicating that practically no polydextrose is absorbed after oral administration. Accordingly, most (~60%) of the administered dose is expelled with feces. Approximately 21% of dose was recovered as $^{14}CO_2$. Since injected polydextrose resulted in very little exhaled radioactivity, the formation of $^{14}CO_2$ in these studies clearly implicates the bacterial flora as being responsible for the partial degradation of polydextrose.

Ingested polysaccharides which are not split by digestive enzymes are not absorbed. These compounds reach the lower gut where they may serve as substrates to the bacterial flora. From the standpoint of benefit to the mammalian host, the microorganisms elaborate two kinds of products, which are distinguished from each other by their capacities to function as caloric sources. Carbon dioxide generated by the bacteria is of no nutritional value, some of it is expelled as flatus and some is absorbed into the bloodstream, transported to the lung, and exhaled. Other microbial metabolites such as volatile fatty acids are absorbed by the host and utilized as a source of energy. Since the animal converts most of these molecules to CO_2, the caloric utilization can be estimated by measuring exhaled $^{14}CO_2$ after ingestion of [^{14}C]polydextrose. As shown by experiments with labeled glucose and acetate, approximately 60% of [^{14}C]glucose or acetate radioactivity is absorbed and utilized and exhaled as $^{14}CO_2$ within the first 24 h. However, in the case of polydextrose, $^{14}CO_2$ measurements can lead to a high estimate of caloric utilization. In rat experiments the animals were housed in special containers that ensured the total collection of $^{14}CO_2$; that obtained from breath as well as flatus. Flatus $^{14}CO_2$ is not caloric. Breath $^{14}CO_2$ is mostly mammalian enzyme derived (caloric) plus some fraction of $^{14}CO_2$ obtained from gut bacterial fermentation that was not expelled as flatus but was absorbed from the gut (not caloric).

The rat experiments show that 21% of orally administered radioactivity was recovered as $^{14}CO_2$. As described above, this quantity represents the sum of CO_2 formed directly by gut flora and of CO_2 resulting from the utilization by the rat of those microbial metabolites (i.e., VFA) which are absorbed from the lower intestines. Because CO_2 of microbial origin cannot be distinguished from that elaborated by mammalian enzymes, an estimate of caloric utilization based on total CO_2 collected obviously is high. Since only 60% of the material utilized by rats is expected to be exhaled, a *maximum* caloric value of 35% is computed as available to the rat under the test conditions with polydextrose. In reality, however, the true caloric value of polydextrose in the rat is less than 35% and is undoubtedly closer to 25% (approximately 1 cal/g), the value obtained from human studies in which only breath $^{14}CO_2$ was collected (Figdor and Bianchine, 1981).

Recognizing the pivotal role the intestinal microflora plays in the metabolic fate of polydextrose, we addressed additional questions. It was shown that polydextrose derived volatile fatty acids are indeed present in feces of the rat. Not all the polydextrose is fermentable by gut flora since polydextrose recovered from rat feces and re-

administered to rats yields less than $^1/_3$ the $^{14}CO_2$ obtained from the first passage through the rat. Adaptation of the intestinal flora to the polysaccharide does not occur. Rats that were prestressed for more than 90 days with polydextrose showed no significant change in metabolic utilization.

As predicted from absorption (persorption) studies of macromolecules, a small amount of intact polydextrose is expected to be absorbed and excreted in urine. Analysis of urinary radioactivity after [^{14}C]polydextrose indicates that a maximum of 0.2% of an orally administered dose is absorbed by the rat.

ACKNOWLEDGMENT

We thank Virgilio Bulanhagui, Lydia Harran, and Nina Odryna for their excellent technical assistance. We are especially indebted to Dr. M. Schach von Wittenau for his advice and guidance throughout this program.

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Received for review February 19, 1981. Revised manuscript received July 30, 1981. Accepted August 18, 1981.

Photoprotection of the Red Pigments of *Monascus anka* in Aqueous Media by 1,4,6-Trihydroxynaphthalene

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The photobleaching of the *Monascus anka* red pigments *N*-glucosylrubropunctamine (**3**) and *N*-glucosylmonascorubramine (**4**) by sunlight irradiation in aqueous solutions at pH 2.8 and 6.0 is substantially inhibited in the presence of 1,4,6-trihydroxynaphthalene (**10**). This protective effect is specific with respect to the number and position of the hydroxyls in the naphthalene ring and seems related to the ability of **10** to form molecular complexes with **3** and **4**.

Fermentation processes for the production of natural products from microorganisms are central to the needs of the pharmaceutical and food industries in terms of drugs, additives, nutrients, and processing aids (Arima, 1977; Woodruff, 1980).

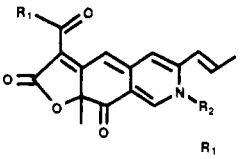
The genetic manipulations of useful microbes through the induction/selection of mutants and the use of DNA recombination techniques (Elander, 1980) will continue to improve the capabilities and efficiency of fermentations.

Against such promising technological background, the available information of the potential of microorganisms as color producers contrasts as fragmentary and superficial. A recent compilation of microbial metabolites (Laskin and Lechevalier, 1973) lists pigmented products whose color, however, is secondary to some significant bioactivity, as in the case of antibiotics, toxins, carcinogens, fungistatics, antitumor compounds, etc. The systematic search for nontoxic, bioinert microbial pigments that could function as food additives is yet to be realized.

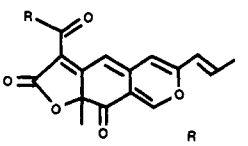
Corporate Research & Development, The Coca-Cola Company, Atlanta, Georgia 30301 (J.G.S., M.C.E.-V., and G.A.I.), and Department of Agricultural Chemistry, Hokkaido University, Sapporo, Japan (H.S. and S.S.).

One important application for food colors is the area of liquid formulations like carbonated beverages, canned fruit juices, and the like. For that end, it is particularly important to establish their colorfastness in solution, as affected by light, pH, and temperature.

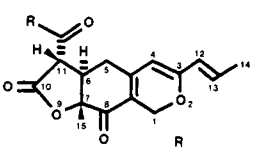
In this paper we discuss our observations on the protection of the red pigments rubropunctamine (**1**) and



	R_1	R_2
1 Rubropunctamine	$n\text{-}C_5H_{11}$	H
2 Monascorubramine	$n\text{-}C_7H_{15}$	H
3 N-glucosylrubropunctamine	$n\text{-}C_5H_{11}$	glucose
4 N-glucosylmonascorubramine	$n\text{-}C_7H_{15}$	glucose



5 Rubropunctatin $n\text{-}C_5H_{11}$
6 Monascorubrin $n\text{-}C_7H_{15}$



7 Monascin $n\text{-}C_5H_{11}$
8 Ankaflavin $n\text{-}C_7H_{15}$

monascorubramine (**2**) against sunlight fading in solution.

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