

Table III. Methods of Addition: Spectrophotometric Determination of Glycyrrhizic Acid in Licorice ($n = 2$)

amount of glycyrrhizic acid added, mg/g	amount of glycyrrhizic acid obtained, mg/g	% recovery
none	1.30	
0.2	1.50	100.0
0.4	1.70	100.0

Table IV. Product Survey ($n = 2$)

product description	amount of glycyrrhizic acid, mg/g
candy wafers (black)	0.11
jelly rings (black)	0.19
licorice toffees	0.88
licorice candies (France)	1.65
jelly candy (black)	0.25
hard-coated licorice candies	0.06
licorice gumdrops	1.39
licorice bits	0.94

The previously described HPLC method was tested on a black licorice with additions at five different levels. Additions were made prior to extraction. Table II summarizes this data. Concentrations of glycyrrhizic acid over a 20-fold range, from 0.5 to 10 mg/mL, were injected onto the LC and found to be linear with a regression coefficient of 0.99.

Additionally, the spectrophotometric method of Cundiff (1964) was evaluated with recovery checked at two levels and an average recovery of 100%. These data are summarized in Table III. A comparison of the HPLC and spectrophotometric methods shows excellent agreement.

Table IV outlines the results from a product survey consisting of eight licorice-containing products which covered the range of licorice candies from gumdrops to hard candies.

SUMMARY

The HPLC technique now allows the routine analysis of glycyrrhizin in licorice products. The technique is rapid, accurate, and precise and can be applied to a variety of licorice-containing confectionery items. Additionally, for those laboratories that do not possess an HPLC, the spectrophotometric method, while not as fast, serves as a good alternative for the analysis of glycyrrhizin in licorice confectionery items.

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

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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 of polydextrose is excreted 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 (VFA) and CO_2 ; the VFA are caloric to the host, but the CO_2 is not. The metabolism and disposition of polydextrose in man is the same as that in the rat. 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.

A substantial portion of the U.S. adult population is overweight (Bray, 1979), and methods and diets designed to normalize the weight of this population are legion. Among the more pleasant and alternative approaches to weight reduction would be the availability of a broad

spectrum of prepared foods with reduced caloric density. The ingestion of such foods would result in a normal volume intake that contains significantly fewer calories when compared to standard food items.

Polydextrose, which was recently approved by the Food and Drug Administration, was developed to fulfill this purpose in foods. It is a substance which contains only 1 cal/g, or approximately 25% the value of food carbohydrate, and has broad utility as a bulking agent in a

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variety of prepared foods. Polydextrose may serve as a total or partial replacement for sugar and as a partial replacement for fat. Thus, the ingestion of baked products, candies, ice creams, puddings, salad dressings, etc. prepared with polydextrose results in a net decline of caloric intake of significant magnitude.

While it is nonsweet, this reduced-calorie bulking agent can provide the bulk and texture that is normally obtained from sugar in many food systems. It is prepared by the melt polycondensation of glucose with small amounts of sorbitol and citric acid—all common food ingredients (Rennhard, 1973). In appearance it is an amorphous, bland tasting powder that is very soluble in water, and it provides solutions that are weakly acidic but otherwise very similar in physical properties to sugar solutions.

Chemical degradation studies and ^{13}C NMR analysis have shown that the polymer contains all possible types of glucosidic linkages between glucose units. It is this random bonding that is primarily responsible for the resistance of the polysaccharide to enzymatic attack, with consequent reduced caloric availability [Rennhard, 1981; also Figdor and Rennhard (1981)]. While most of the polymeric mixture that makes up polydextrose has a sufficiently high molecular weight so that it is resistant to enzymatic and microbial attack, the upper limit molecular weight is held to less than 20 000, which allows rapid excretion of the trace amounts of polysaccharide that may be absorbed from the intestine (Arturson and Wallenius, 1964; Arturson et al., 1971). This control of the molecular weight range also allows the product to have the appropriate physical-chemical characteristics necessary for a material designed to replace sugar.

Polydextrose has been shown to be suitable for the diabetic in that it does not raise serum glucose values or create an insulin demand (McMahon, 1974; Bachmann et al., 1982). Preliminary research also suggests that polydextrose is noncariogenic as well (Mühlemann, 1980).

The synthesis and characterization of polydextrose have been reported by Rennhard (1973, 1981), food applications were discussed by Torres and Thomas (1981), and the caloric utilization, metabolism, and disposition of polydextrose in the rat were described by Figdor and Rennhard (1981). A general review of polydextrose was presented by Beereboom (1979).

The purpose of this paper is to discuss the techniques and the results that led to the conclusion that polydextrose has a caloric utilization value in man of 1 cal/g.

EXPERIMENTAL SECTION

Experimental Protocol. After signing informed-consent forms four normal, healthy, male volunteers received 10 g of nonlabeled polydextrose incorporated into a chocolate milk drink immediately after breakfast, daily, for 7 days. On the eighth day each received the standard 10-g portion containing 72 μCi of uniformly labeled [^{14}C]polydextrose. On the 2 subsequent days after receiving the labeled material, each subject continued to receive 10 g of nonlabeled polydextrose immediately after breakfast. Prior to receiving the labeled dose each patient submitted a urine and fecal collection and gave a 4-min breath collection (see below).

Sample Analysis. *Breath $^{14}\text{CO}_2$.* Each subject breathed 30 L of expired air into a large rubber bag over a carefully timed 4-min period, thus exhaling at the normal "average" rate of approximately 7.5 L of expired air/min. The contents of the bag was slowly bubbled through 65 mL of Hyamine (New England Nuclear) and the Hyamine was assayed for radioactivity. Breath collections were obtained at the following intervals after the

labeled dose: 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 24, 36, and 48 h.

The total CO_2 content of the Hyamine samples was estimated as follows. Expired breath contains 4.5% (by volume) of CO_2 (Spector, 1956), and the 30 L of expired air from each subject is expected to contain 2363 mg of CO_2 . Since this amount of CO_2 is absorbed in 61 mL of Hyamine, a concentration of 38.7 mg of CO_2/mL Hyamine is to be expected. (Thirty liters L of air was bubbled through an initial volume of 65 mL of Hyamine, and as a result of evaporation of the Hyamine-methanol solvent during the passage of 30 l of air, the final volume of Hyamine is 61 mL.) Total CO_2 content of Hyamine was measured in randomly selected Hyamine samples and quantitated by GLC. An aliquot of the Hyamine was placed in a sealed evacuated flask of known volume and acidified with sulfuric acid. By means of a gas syringe an aliquot of the resulting CO_2 was assayed by GLC and quantitated by comparison with a calibration curve prepared from CO_2 -saturated Hyamine solutions. A Hewlett-Packard GLC 5750 with a thermal conductivity detector was used with a column 6 ft \times 4 mm, coiled glass with Porapak Q, 100–120 mesh, at 100 $^\circ\text{C}$. The inlet port was maintained at 160 $^\circ\text{C}$ and the detector at 160 $^\circ\text{C}$. The carrier gas was helium at 40 mL/min.

Urine. During the first day after [^{14}C]polydextrose administration urine was collected and pooled as follows: 0–6, 6–12, and 12–24 h. Starting with day 2 each 24-h collection was pooled for a total of 7 days of collection. Each sample was immediately frozen at the end of the collection period.

Feces. Feces were immediately frozen, and the time and date of collection were recorded.

Persorption Studies. Procedures used for the isolation and identification of polydextrose in urine have been described earlier (Figdor and Rennhard, 1981).

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. The preparation of [^{14}C]polydextrose has been described (Figdor and Rennhard, 1981).

$^{14}\text{CO}_2$. Aliquots of Hyamine (0.4 mL) were dissolved in 15 mL of 30/70 scintillator (see below) and assayed for radioactivity.

Urine. Aliquots of urine or diluted urine (0.2 mL) were assayed in 30/70 scintillator with added Triton X. 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 (5%) added to Omnifluor dissolved in toluene.

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%.

Volatile Fatty Acids (VFA) in the Intestinal Flora of Man after [^{14}C]Polydextrose. *Analysis of Human Feces.* Three subjects received 10 g [^{14}C]polydextrose containing 69.4 μCi of radioactivity as a water solution. Feces were collected for three or four successive 24-h periods after the radioactive dose, and each 24-h collection was pooled for each subject.

In Vitro Experiments with Human Feces. Incubations were carried out with fresh human feces that were carefully collected in an atmosphere of carbon dioxide to ensure anaerobic conditions. The collection was homogenized in

Table I. Recovery of Radioactivity from Man after Oral Administration of [¹⁴C]Polydextrose

collection	% of administered dose for subject no.				mean ± SD
	1	2	3	4	
¹⁴ CO ₂ ^a	22.00	12.48	13.78	15.62	15.97 ± 4.2
caloric utilization	36.67	20.80	22.97	26.03	26.62 ± 7.0
urine: 0-24 h	0.96	0.80	0.64	0.92	0.83 ± 0.1
24-48 h	0.53	0.26	0.15	0.38	0.33 ± 0.2
48-72 h	0.21	0.07	0.05	0.12	0.11 ± 0.1
72-168 h	0.17	0.10	0.09	0.18	0.14 ± 0.05
total urine, 0-7 days	1.87	1.23	0.93	1.60	1.41 ± 0.4
feces: 0-24 h	1.76	1.72	6.59	0.04	2.53 ± 2.8
24-48 h	15.79	50.23	49.68	42.95	39.66 ± 16.3
48-72 h	17.26	3.09	0.32	3.77	6.11 ± 7.6
72-168 h	5.98	0.51	0.15	0.42	1.77 ± 2.8
total feces, 0-7 days	40.79	55.55	56.74	47.18	50.07 ± 7.5
total: urine, feces, and caloric utilization	79.33	77.58	80.64	74.81	78.09 ± 2.5

^a ¹⁴CO₂ collected for the interval 0-24 h after the labeled dose.

oxygen-free water in an atmosphere of carbon dioxide, and 18 mg (48 μCi) of [¹⁴C]polydextrose was added along with 150 mg of sodium thioglycolate to maintain anaerobic conditions. The vessel was incubated at 38 °C. Aliquots (50 mL) were withdrawn at 4, 22, and 30 h, adjusted to pH 8.5, and lyophilized. Methods for the isolation and identification of volatile fatty acids in feces have been described (Figdor and Rennhard, 1981).

Caloric Utilization. A survey of the literature shows that a total of 60% of an available carbon source, such as [¹⁴C]acetate is exhaled as ¹⁴CO₂ within 24 h (Shreeve et al., 1959; Hellman et al., 1951). This has been confirmed experimentally by the authors. Following the administration of an aqueous solution of uniformly labeled D-[¹⁴C]glucose or [1-¹⁴C]acetate by gavage to rats, 62.3% and 55.4%, respectively, of the radioactivity was recovered as ¹⁴CO₂ within 24 h.

Consequently, for estimation of the total caloric utilization of polydextrose in man, the actually recovered ¹⁴CO₂ is corrected by the catabolic conversion factor (acetate → CO₂) of 0.6 (Figdor and Rennhard, 1981).

RESULTS

A summary of the disposition and recovery of radioactivity from the four subjects after [¹⁴C]polydextrose administration is presented in Table I. The recovery of radioactivity as ¹⁴CO₂ in the breath was 12.5-15.6% of the dose for subjects 2,3, and 4 but somewhat higher, 22%, for subject 1. Conversion of these results into estimated caloric utilization for [¹⁴C]polydextrose indicates that the average value is 26.6% of the dose, or approximately 1 cal/g (see Experimental Section for a discussion of caloric utilization). For reasons discussed below this figure is a high estimate. Figure 1 is a graphic presentation of the average rate of ¹⁴CO₂ recovery from the four subjects.

To show that no significant quantities of ¹⁴CO₂ were lost during the collection procedure, we assayed randomly selected Hyamine samples for total CO₂ content. As discussed under Sample Analysis, approximately 39 mg of CO₂/mL of Hyamine is to be expected. The results of these assays presented in Table II show that the amount of CO₂ trapped is very close to the CO₂ that was expected, with an overall average value of 36 mg of CO₂/mL of Hyamine.

Urinary recovery of radioactivity averaged 1.4% of the dose (Table I). The major portion of excreted label was recovered in the 0-24 h collection interval. As discussed below, greater than 95% of human urinary radioactivity after [¹⁴C]polydextrose administration is not directly attributable to polydextrose and consists of radioactive urea and other normal endogenous metabolism products.

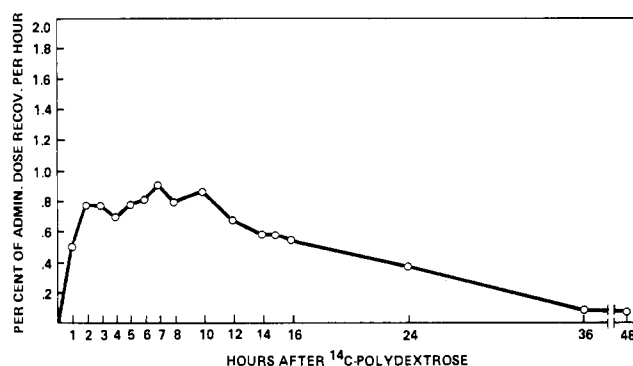


Figure 1. The recovery of ¹⁴CO₂ from the expired breath of man after oral administration of [¹⁴C]polydextrose. Average of four subjects.

Table II. Total CO₂ Content of Hyamine Samples

Hyamine sample, h after labeled dose	mg of CO ₂ /mL of Hyamine ^a for subject no.			
	1	2	3	4
1	40	36	33	45
3	33	28	32	43
5		30	35	42
7	36	33	29	47
10		28	43	
14	34	31		
average	36	31	34	44
overall average			36	

^a On the basis of "normal" values, approximately 39 mg of CO₂/mL of Hyamine is expected. See Experimental Protocol.

Fecal recovery of radioactivity accounted for an average of 50% of the administered dose (Table I), and most of the radioactivity was recovered during the interval of 24-48 h after polydextrose administration. There was good correlation of caloric utilization with fecal excretion. Subject 1, with the higher exhalation of ¹⁴CO₂, showed the lowest recovery of fecal radioactivity. The other three subjects exhibited similar recovery patterns.

The overall recovery or accountability of radioactivity was approximately 80% of dose and did not differ appreciably among the four subjects (Table I). The observed recovery appears to be low for a tracer metabolism experiment but it does not take into account losses of ¹⁴CO₂ as flatus, which was not collected.

A recovery of 80% of the administered radioactivity represents a "loss" of approximately 2 g of polydextrose based on the 10-g dose. This "loss" must have occurred via flatus ¹⁴CO₂, which was not collected in the human

Table III. Persorption of [¹⁴C]Polydextrose in Man after Oral Administration^a (Recovery of Polydextrose from Urine)

	sub- ject 1	sub- ject 2	sub- ject 3	mean
% recovery of administered radioact maximum ^b % of urine radioact that is polydextrose related	0.96	0.80	0.64	0.80
maximum ^b % recovery from urine of administered polydextrose	2.29	1.75	9.06	4.37
	0.022	0.014	0.058	0.03

^a All data are derived from 0–24-h urine collection.

^b The urinary fraction that contains polydextrose also contains normal endogenous radioactive substances derived from radioactive volatile fatty acids produced from polydextrose by fermentation in the lower gut. Thus, conclusions regarding persorbed polydextrose are maximum possible persorbed amounts, since some of this radioactivity is not polydextrose related.

experiment. To demonstrate that such loss is not unreasonable, we cite a relevant experiment with beans. Oligosaccharides found in beans, such as stachyose (a tetrasaccharide), which are not split by digestive enzymes are fermented in the lower intestine. After a meal of baked beans flatus volume has been shown to increase from a basal rate of 15 mL/h to approximately 170 mL/h for an 8-h period of observation. During this time the CO₂ concentration in flatus increased from 8% to 50% (Steggerda, 1968). Thus, appreciable quantities of a saccharide can be lost as flatus CO₂.

Absorption of Intact Polydextrose from the Gut. It has been shown that small quantities of a variety of macromolecular substances may cross the intestinal wall and become absorbed into the general circulation. Ferritin (Bockman and Winborn, 1965, 1966), horseradish peroxidase (*M_r* 40 000) (Warsaw et al., 1971), starch granules (Volkheimer et al., 1968), and partially degraded carageenan (*M_r* 20 000) (Abraham et al., 1972) are but a few of the many substances persorbed intact from the gut.

Since less than 1% of the administered [¹⁴C]polydextrose radioactivity was recovered within 24 h in urine from the four subjects described in this report, it was important to determine how much, if any, of this urinary radioactivity was polydextrose. It has been shown that in rats intravenously administered polydextrose is rapidly and completely cleared into urine (Figdor and Rennhard, 1981). Thus, a determination of the polydextrose content of urine after oral administration is a reliable estimate of the extent of persorption of polydextrose from the gut.

Urine collected from subjects 1, 2, and 3 during the 0–24-h interval after [¹⁴C]polydextrose administration was examined for the presence of polydextrose. The results of those assay procedures are shown in Table III and show that less than 5% of urinary radioactivity is polydextrose related. On the basis of an average urinary recovery of 0.8% of the dose (Table I) these data indicate that a maximum of approximately 0.03% of fed [¹⁴C]polydextrose is persorbed through the intestinal epithelial wall.

DISCUSSION

For all practical purposes, polysaccharides are not absorbed as such from the gastrointestinal tract. They first must be hydrolyzed to monomers which then may pass into the blood stream. Following absorption, the sugar is utilized to produce energy by a series of enzyme-specific biochemical reactions which produce substantial quantities of CO₂, most of which is exhaled immediately. It has been

shown in this laboratory that approximately 60% of radioactivity is recovered as ¹⁴CO₂ from rats within 24 h after ingestion of [¹⁴C]glucose. The digestive enzymes secreted by mammals are quite specific in that they split some (starch) but not other (cellulose) carbohydrates. Therefore it is not surprising that many carbohydrates present in food reach the lower intestine. Here they are exposed to the catabolic activity of the microflora and may serve as substrates for bacterial enzymes.

Bacterial digestion of polysaccharides in the gastrointestinal tract can make a contribution to the nutritional economy. If the microorganisms are capable of utilizing the carbohydrate present, metabolites such as CO₂, alcohols, and organic acid are elaborated. The CO₂ formed is rapidly lost via breath and flatus; with respect to the host it is noncaloric. Many of the other small organic molecules are absorbed by the host and utilized. They are a source of energy to the animal, which converts these compounds mostly to CO₂. Because approximately 60% of radioactivity is recovered from breath as ¹⁴CO₂ in the first 24 h, measurement of exhaled CO₂ indicates how much of a food ingredient such as polydextrose has provided energy to the animal. The energy may be derived from the absorption of monomers or as a consequence of microbial production of organic molecules which are utilized by the host. However, some of the CO₂ exhaled is produced directly by the microflora, absorbed into the blood stream, and transported to the lung. Consequently, an estimate of caloric utilization based on collected CO₂ will define the maximum possible value only.

The experiment in man described in this communication was carried out to compare the metabolism of polydextrose in man with that in rats and to quantitate the caloric utilization of polydextrose by man.

The disposition of polydextrose by rats has been elaborated in a series of experiments (Figdor and Rennhard, 1981). Injected polydextrose is metabolically inert and very rapidly excreted unchanged with urine. Orally administered polydextrose is practically not absorbed, and most of the product is expelled with feces. Significant quantities, however, are degraded by the gut flora, which produces compounds normally present in the intestine. Radioactive volatile fatty acids were isolated from rat feces after [¹⁴C]polydextrose administration. In addition, 5 h after a dose of [¹⁴C]polydextrose, 58% of the administered radioactivity was recovered from the cecum, and 13% of the administered radioactivity was isolated from cecum contents and shown to be ¹⁴C-labeled volatile fatty acids (Figdor and Rennhard, 1981). Some of these microbial metabolites, such as the volatile fatty acids, are absorbed by the rat, which metabolizes these compounds further, largely to CO₂. This latter process, the metabolism by the rat of polydextrose-derived bacterial products, provides energy to the animal.

Similarly, feces collected from man after the ingestion of [¹⁴C]polydextrose were shown to contain ¹⁴C-labeled volatile fatty acids (Table IV). Furthermore, the *in vitro* incubation of [¹⁴C]polydextrose with human feces resulted in the conversion of 17% of the added radioactivity to ¹⁴C-labeled volatile fatty acids during the 22 h of incubation (Table V). Thus, as was shown in the rat polydextrose is fermented by the microflora in the human lower intestinal tract, resulting in the production of volatile fatty acids.

It is important to recognize the design of this human metabolism study. While in the rat experiment *all* CO₂ generated from polydextrose was measured, regardless of its mammalian or bacterial origin, in the human study

Table IV. Total Volatile Fatty Acids in Human Feces after [¹⁴C]Polydextrose

subject	day	% of fecal radioact
E.B.	1	1.1
	2	1.8
	3	1.6
L.S.	av	1.5
	1	4.3
	2	1.0
W.W.	3	1.1
	av	2.1
	1	2.3
	2	0.6
	3	0.9
	4	0.9
	av	1.3
	grand av	1.6

Table V. Volatile Fatty Acids Formed by Fermentation of [¹⁴C]Polydextrose with Human Fecal Microflora

duration of fermentation, h	[¹⁴ C]-VFA, %
4	8.2
22	17.2
30	17.5

exhaled CO₂ only was determined; bacterially generated CO₂ was mostly lost as flatus. Consequently, the caloric utilization calculated from the rat data is too high, but the recovery of total radioactivity was good (Figdor and Rennhard, 1981) and of course better than in the human experiment. However, the calculated caloric utilization from the human experiment is still probably somewhat high due to the inclusion in the breath of microorganism-generated CO₂. The metabolism and disposition of polydextrose in mammals (man and rat) are summarized and depicted in Figure 2

SUMMARY

Polydextrose is a low caloric nonsweet bulking agent prepared by the random polymerization of glucose and small amounts of sorbitol and citric acid. Because it is a randomly linked polysaccharide it is resistant to mammalian enzymes. It is, however, partially susceptible to degradation by the enzymes of the gut microflora.

After oral administration of [¹⁴C]polydextrose to man, negligible amounts, less than 1.5% of the administered radioactivity, was recovered in the urine. Analysis of urinary radioactivity after [¹⁴C]polydextrose indicates that a maximum of 0.03% of an orally administered dose is absorbed by man.

The major portion of the administered dose is expelled with the feces, a fraction of fed polydextrose undergoes fermentation in the lower G.I. tract to CO₂ and small organic molecules such as volatile fatty acids. The microflora-derived CO₂ is not caloric to the host and is lost via breath and flatus. The small organic molecules are absorbed, are caloric to the host, and are eventually expelled as CO₂ in the breath.

Approximately 16% of the administered polydextrose radioactivity is recovered from the breath as ¹⁴CO₂, indicating a maximum caloric utilization of approximately 25% or 1 cal/g. This is a high estimate since breath contains microbial-derived CO₂ (noncaloric) and small organic molecule derived CO₂ (caloric).

The pathways and mechanisms of polydextrose metabolism and disposition that have been demonstrated in man are the same as those previously elucidated in the rat (Figdor and Rennhard, 1981).

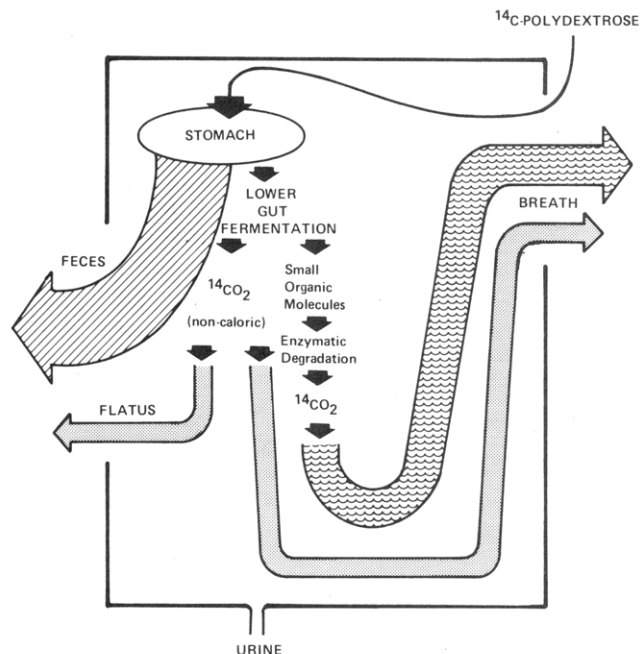


Figure 2. Summary of the metabolism and disposition of [¹⁴C]polydextrose in man and rat.

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