Intestinal metabolism of a random-bonded polyglucose bulking agent in humans: In vitro and in vivo studies of hydrogen evolution

NOEL W. SOLOMONS and ANDREA ROSENTHAL

CAMBRIDGE, MASSACHUSETTS, and GUATEMALA CITY, GUATEMALA

In vivo and in vitro experiments were conducted to determine the extent of intestinal fermentation of polydextrose, a random-bonded glucose polymer proposed as a low-calorie bulking agent in weight-reducing diets. The evolution of hydrogen gas was the index of bacterial fermentation. Oral ingestion of 15 gm polydextrose by healthy volunteers produced a flat breath hydrogen response, equivalent to that of glucose, and significantly less than that of lactulose. In vitro incubation of a polydextrose solution with fecal homogenates produced 24.8% of the hydrogen production of a comparable glucose solution. When either milk or lactose-hydrolyzed milk containing 18 gm intrinsic carbohydrate was mixed with 18 gm polydextrose, a significantly greater breath hydrogen excretion was observed as compared with the respective beverages alone. There is minimal in vivo fermentation of polydextrose when consumed alone, but when mixed into foods it may produce carbohydrate malabsorption or itself be more readily fermented. (J Lab Clin Med 105:585-592, 1985.)

Control of body weight is important not only for aesthetic reasons, but because it has been demonstrated that excess weight is associated with increased morbidity and mortality.[1] Innumerable strategies for the reduction and maintenance of weight within desired limits have been devised with variable short- and long-term success. One of the new strategies for weight control is the principle of dietary dilution with "nonavailable" calories. It is based on the principle that psychologic gratification and physiologic satiety can be achieved with meals containing "bulking" substances that dilute the energy density without themselves adding calories.

The most basic approach uses diets enriched with dietary fiber. The biologic potentials and limitations of this strategy have been reviewed by Van Itallie,[2] but a major cultural drawback to fiber-enriched diets is organoleptic; taste preferences of Western populations favor sweet and highly refined diets. To circumvent this problem, food technologists have provided a number of synthetic substances aimed at maintaining the familiar and appealing flavors and textures of foods while replacing part of the carbohydrate and fat with compounds of substantially lower energy content. Two such compounds are sucrose polyester[3,4] and random-bonded polyglucose polymers.[5,6]

A new product marketed commercially as polydextrose (Pfizer Inc., New York, N.Y.) is derived from polymerization of glucose in such a way that polymers of up to 20,000 daltons containing all possible carbon-carbon bonds and branching configurations are formed. Although pancreatic and salivary α-amylases might be expected to hydrolyze the α-1-4 glycosidic linkages of the polymers, such unions represent only a fraction of the bonds; substantial further hydrolysis of the molecules in the small bowel would be improbable, and the compounds would pass into the large bowel. Similarly, although colonic bacteria might elaborate enzymes capable of digesting additional glycosidic linkages, such as β-1-4, and α-1-6, only a modest net liberation of

From the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, and the Institute of Nutrition of Central America and Panama, Guatemala City.

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Reprint requests: Noel W. Solomons, M.D., International Nutrition Program, M.I.T., Rm. 20A-201, 18 Vassar St., Cambridge, MA 02139.

monosaccharide units would become available for fermentation in the large bowel. Initial studies in rats[5] and humans[6] have been reported that used polydextrose with ^{14}C-labeled glucose residues incorporated into the polymers as metabolic markers. Because the fermentative liberation of gaseous H_2 in vitro and in vivo is another index of bacterial metabolism of carbohydrates,[7-9] we examined the intestinal metabolism of polydextrose by in vitro incubation and the H_2 breath-analysis test in healthy volunteers.

METHODS

A total of 27 healthy adult volunteers participated in the studies. Sixteen were from the university campus of Massachusetts Institute of Technology and 11 from the professional and technical staff of the Institute of Nutrition of Central America and Panama. They ranged in age from 19 to 45 years, and included 15 women and 12 men. None of the volunteers was taking or had recently taken antibiotics orally. None had any active or recent history of gastroenteritis or a diagnosis of any chronic gastrointestinal disorder. All agreed to participate after the nature, purpose, and risks of the procedures had been explained and informed consent obtained. The protocol had received prior approval from the Committee on the Use of Humans as Experimental Subjects of M.I.T.

Breath collection and analysis. Samples of mixed expired air were collected by having the volunteers breathe through a low-resistance, one-way Hans Rudolph valve into a 5 L rubber anesthesia gas bag. A 60 ml aliquot was transferred immediately to a plastic syringe fitted with a three-way stopcock, and analyzed within 8 hours. Breath H_2 concentration, expressed in parts per million, was determined on a gas chromatograph (Microlyzer model 12; Quintron Instrument Co., Milwaukee, Wis.) calibrated with a standard reference gas mixture containing 100 ppm H_2 in nitrogen (Scotty Gas II; Supelco, Bellefonte, Pa.).[10,11]

In vivo experimental procedures. In experiment 1, seven individuals were given 100 ml aqueous solution containing 15 gm carbohydrate substrate on three occasions. Doses of glucose, lactulose (Cephulac; Merrill-National, Cincinnati, Ohio), a nonabsorbable disaccharide, and polydextrose (Pfizer) were presented in random order with at least a 72-hour interval between studies. Breath samples were collected initially and at 30-minute intervals after the administration of the dose for 5 hours. Three volunteers participated in a derivative protocol in which breath samples were collected before and for 10 hours after an orally administered dose of 15 gm polydextrose at 30-minute intervals.

Two individuals participated in experiment 2, to determine the blood-glucose responses to polydextrose. Each received 25 gm glucose in 100 ml water on one occasion, and 25 gm polydextrose in the same volume of water on the subsequent day. Breath H_2 was analyzed at 30-minute intervals over 5 hours. A fasting blood sample and postdose blood samples at 30, 60, 90, and 120 minutes were collected. Blood glucose concentrations were determined by an *o*-toluidine method.[12]

Two additional breath H_2 studies were performed in two cohorts of seven volunteers each. Experiment 4 involved seven proved lactose-absorbers studied on two occasions, once after ingestion of 360 ml intact milk (containing 18 gm lactose) alone, and a second time after ingestion of the same volume of milk to which 18 gm polydextrose had been added. Breath samples were collected before and at 60-minute intervals after the dose for 6 hours. Experiment 5 involved a 360 ml dose of lactose-hydrolyzed milk. The milk was treated for 24 hours at 4° C with 5 drops of a commercial β-galactosidase ("lactase") of yeast origin (LactAid; LactAid Co., Pleasantville, N.J.). This treatment hydrolyzes >90% of the lactose to glucose and galactose.[13] Seven subjects were studied on two occasions, once after drinking the lactose-hydrolyzed milk alone, and again after 18 gm polydextrose had been mixed into the hydrolyzed milk. The breath collection procedure was identical to that in experiment 4.

In vitro experimental procedures. In vitro incubations of carbohydrate with fecal homogenates were conducted in stool samples from eight subjects by modifications of the methods reported by others.[14,15] Six gm fresh stool was homogenized in 18 ml 0.1 mol/L potassium phosphate–buffered saline solution, pH 7.4. Two ml aliquots of the stool homogenates were placed in the bottom of each of seven 20 ml glass tubes (Becton, Dickinson and Co., Oxnard, Calif.). The tubes were stoppered with rubber caps, and 2 ml of a 0.625% solution of carbohydrate solution (0.625 gm glucose or polydextrose powder in 100 ml buffer) were injected into six tubes, three with glucose and three with polydextrose. A seventh tube injected with buffer alone served as a control for spontaneous fermentation of intrinsic carbohydrates in the stool. The tubes were placed in an agitator-type water bath at 37° C and incubated for 60 minutes. At the conclusion of the incubation, 40 ml of the headspace gas was collected from the tube by displacement with water. The gas was analyzed for H_2 concentration with the Microlyzer model 12. If the initial H_2 concentration in the headspace gas was beyond the linear range of the H_2 analyzer, samples were serially diluted with room air until the concentration could be registered on the instrument. The efficiency of in vitro fermentation of polydextrose was expressed as a proportion of the H_2 production in the companion tubes containing glucose.

Data analysis. A rise in breath H_2 concentration of ≥15 ppm during the collection period after the oral administration of a dose of carbohydrate solution was considered to signify complete absorption of substrate, and an increment of ≥20 ppm was considered to represent biologically significant fermentation of carbohydrate in the colon. This criterion is derived by analogy to the breath H_2 threshold for defining lactose malabsorption after a dose of lactose solution or milk,[16-18] and has been used by some to define malabsorption of the carbohydrates in a mixed meal.[19] Intragroup comparisons of the results of different treatments were analyzed by the paired t test.[20]

RESULTS

Fig. 1 illustrates the individual data for breath H_2 responses to 15 gm orally administered glucose and

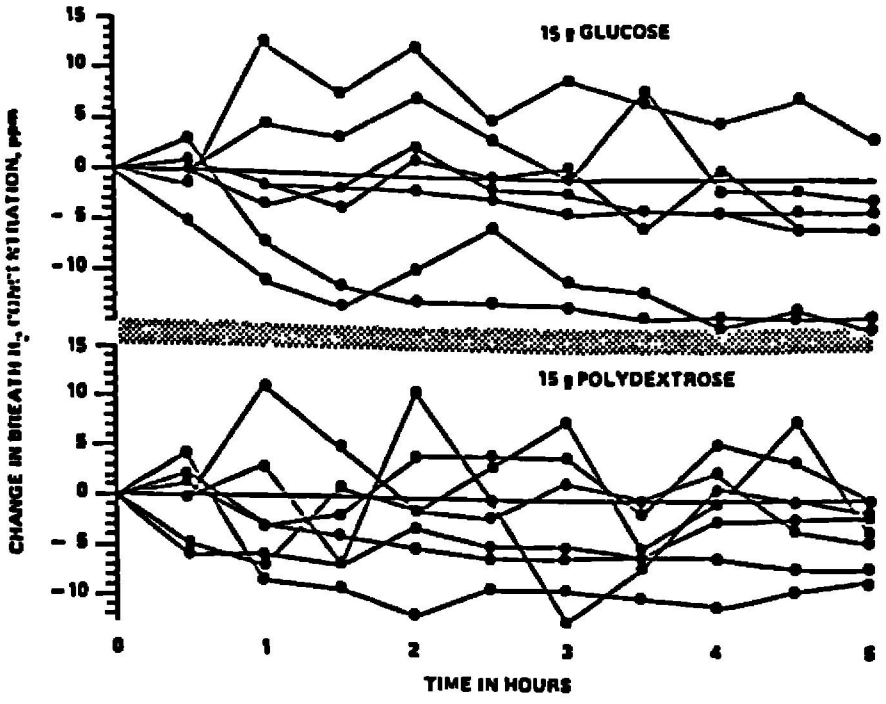

Fig. 1. Individual curves of change in breath H_2 concentration at 30-minute intervals over 5 hours in seven healthy volunteers who consumed aqueous solutions containing 15 gm glucose and 15 gm polydextrose in experiment 1. In no instance was there any increment of >15 ppm above fasting breath H_2 level.

polydextrose in the seven volunteers studied for 5 hours in experiment 1. As demonstrated, no individual had an increment in H_2 concentration >15 ppm. The composite mean increment for the serial changes in breath H_2 in response to the two carbohydrates is shown in Fig. 2, and once again no differences in mean H_2 excretion between the two treatments were observed at any postdose interval. This contrasts with the results with the nonabsorbable sugar lactulose, which showed an increment in breath H_2 concentration in each individual of at least 20 ppm (individual data not shown), and the composite curve of the rise in breath H_2 showed significant differences from the responses to glucose and polydextrose at almost all sampling intervals (Fig. 2). When the collection period after the 15 gm orally administered dose of polydextrose was continued for an additional 5 hours to 10 hours postdose, the maximum increments with respect to basal breath H_2 concentration for the three participants were 4, 5, and 4 ppm, suggesting that a delayed appearance of H_2 was not present (data not shown).

Data from the two individuals participating in the simultaneous plasma glucose and breath H_2 studies (experiment 2) after the ingestion of 25 gm glucose or polydextrose are shown in Fig. 3. Neither had a rise in breath H_2 >15 ppm with either carbohydrate. The oral glucose but not the polydextrose produced an increment in blood glucose concentration >20 mg/dl.

Data from the in vitro incubation studies with fecal homogenates from healthy volunteers (experiment 3) are shown in Fig. 4. Four of the donors had previously taken a single dose of polydextrose orally, and four had not. Compared with an equivalent amount of glucose, the relative efficiency of fermentation of polydextrose ranged from 3.8% to 44.8% (mean ± SEM 24.8% ± 5.4%).

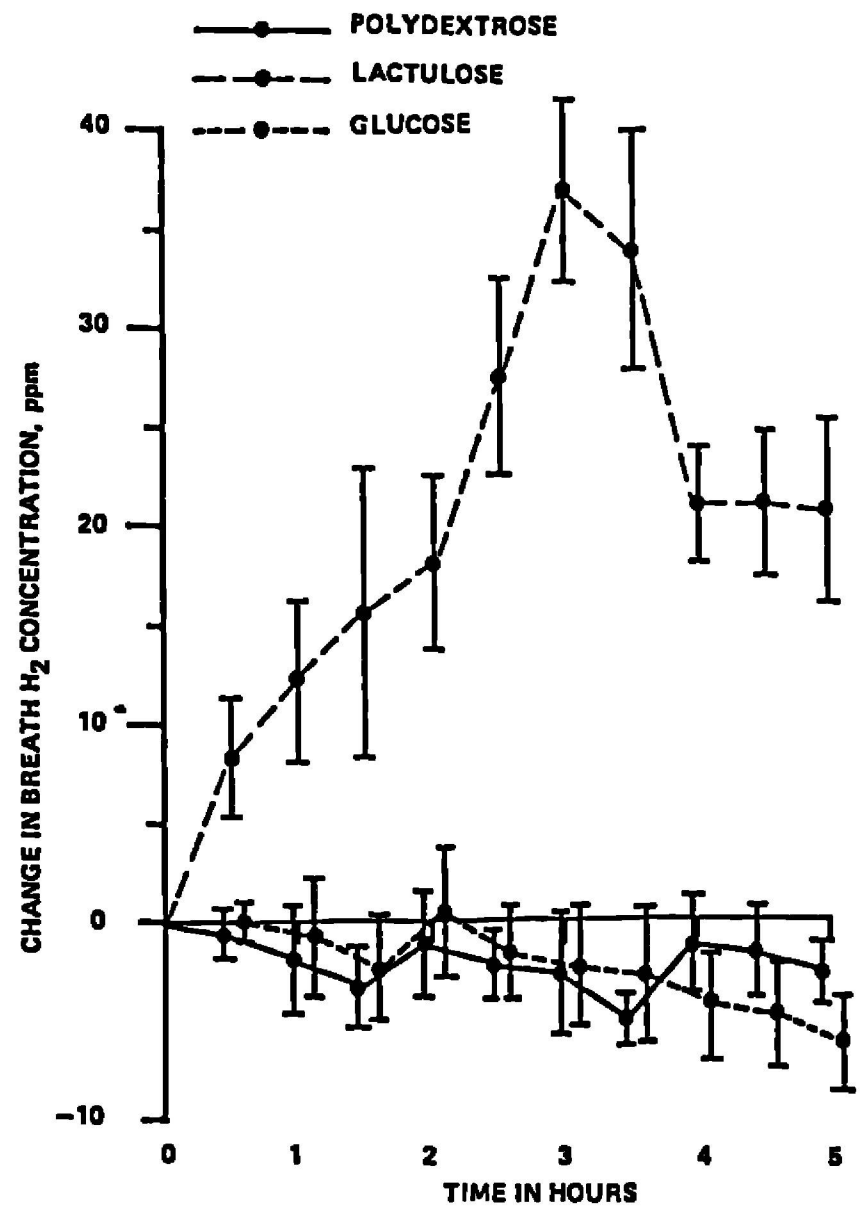

Fig. 2. Composite curves of mean 30-minute interval changes in breath H_2 concentration in seven healthy volunteers in experiment 1 who consumed aqueous solutions containing 15 gm glucose, 15 gm polydextrose, and 15 gm nonabsorbable disaccharide, lactulose; each subject performed each of three breath tests.

The individual breath H_2 curves in response to 360 ml milk alone or with 18 gm polydextrose added in three of seven lactose-absorbers participating in experiment 4 are shown in Fig. 5. A range of variability between the two tests is shown. The composite mean for the breath H_2 response and the peak increment in breath H_2 (Fig. 6) showed a greater H_2 production with the milk plus polydextrose, and the maximum increment in breath H_2 was consistently greater with the glucose polymer added. Two of seven who absorbed lactose had a rise of >20 ppm in breath H_2 with the combination of whole milk and polydextrose.

Even when the milk was preincubated with β-galactosidase to hydrolyze the lactose into its monosaccharide constituents before ingestion (experiment 5), a similar pattern of a greater breath H_2 response to the beverage containing polydextrose, greater peak H_2 increments, and an incidence of positive breath tests (three of seven subjects) was observed (Fig. 7).

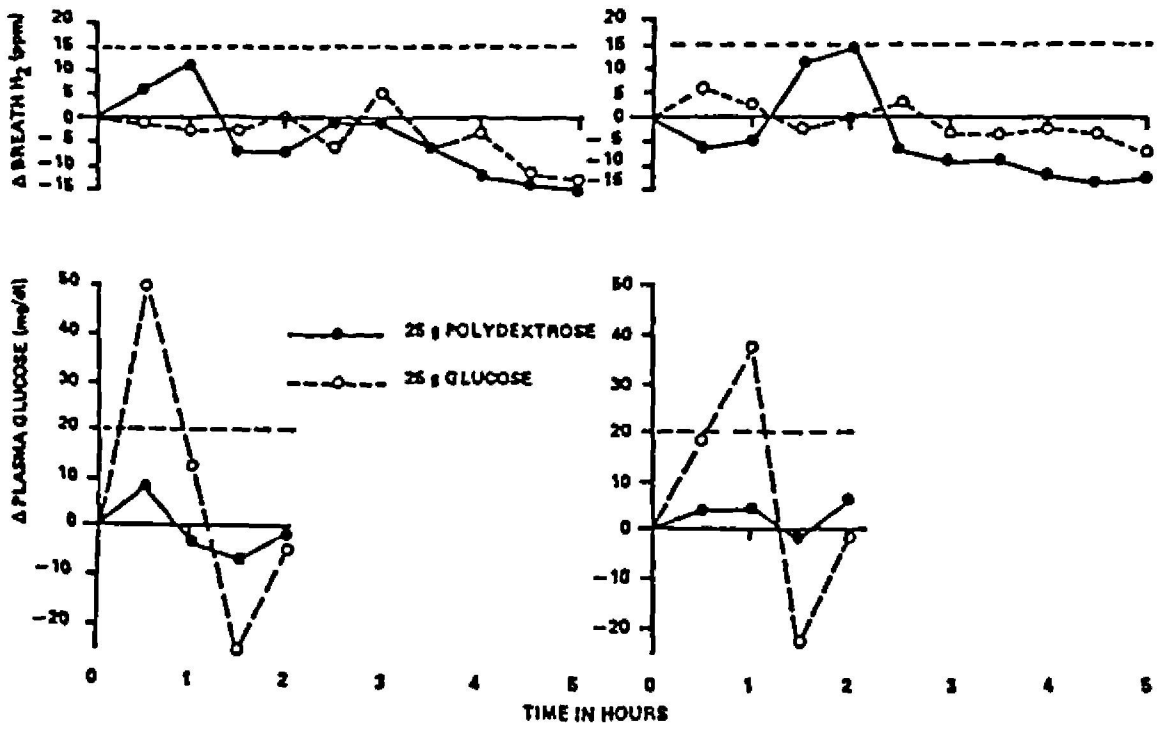

Fig. 3. Upper graph shows change in breath H_2 concentration in two healthy men at 30-minute intervals over 5 hours after ingestion of aqueous solutions containing 25 gm glucose or 25 gm polydextrose in experiment 2. With neither substrate was there an increment of ≥15 ppm above fasting breath H_2 level Lower graph shows change in plasma glucose concentration in same subjects at 30-minute intervals over 2 hours after ingestion of respective test solutions. Only with glucose treatment was a significant increase in plasma glucose concentration observed

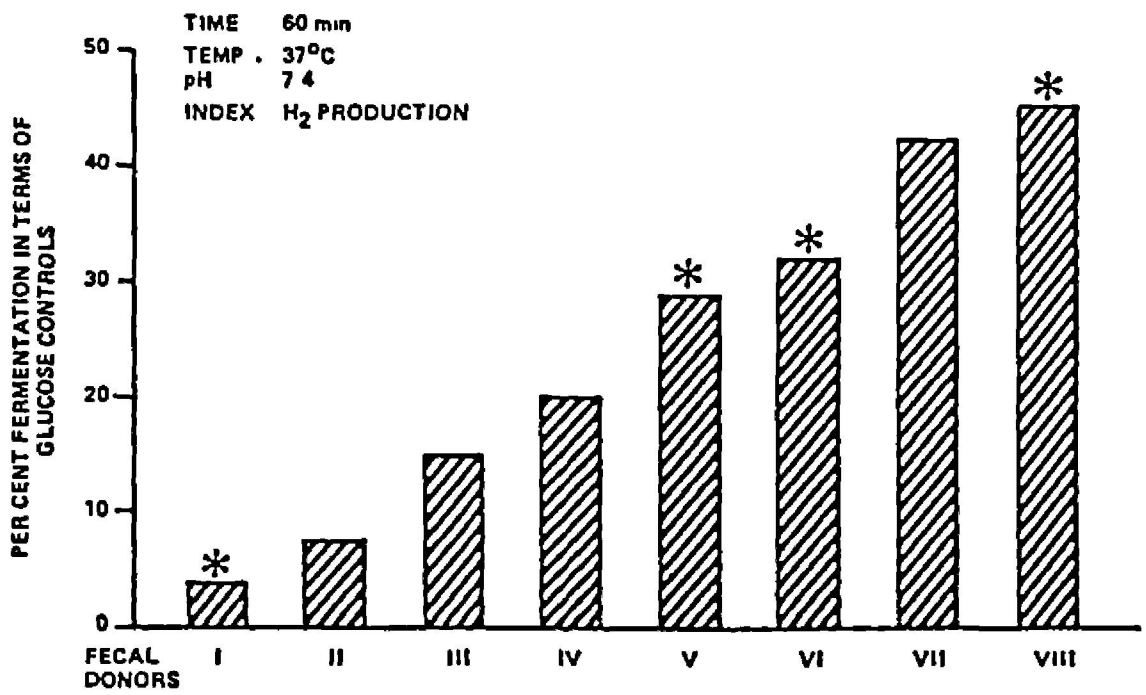

Fig. 4. Efficiency of in vivo fermentation of polydextrose by fecal homogenates incubated under specified conditions for eight individuals in experiment 3 Results are expressed as percentage of total volume of H_2 evolved in simultaneous incubation with similar concentration of glucose *, Four individuals previously exposed to polydextrose orally administered

No formal procedure for collecting information on gastrointestinal tract symptoms was included in the protocol. Nevertheless, although no subjects with the aqueous 15 gm dose of polydextrose (experiment 1) complained of symptoms, several participants in the milk/polydextrose trials (experiments 4 and 5) spontaneously informed the testers of the occurrence of meteorism and flatulence after the ingestion of the test beverage. No subject reported the passage of a liquid stool in response to any of the treatments in the entire study

DISCUSSION

That H_2 is liberated in the process of bacterial fermentation in the intestine[8,9] led us to adopt an alternative approach to evaluate the intestinal metabolism of polydextrose in humans, one that did not require the use of radioisotopes: the H_2 breath-analysis test Lactulose represents a standard of a nonabsorbable but completely and rapidly fermentable, sugar[21] Glucose is also fermentable, but it is generally so efficiently absorbed in the proximal intestine by normal persons that ingestion of glucose elicits no appreciable H_2 ex-

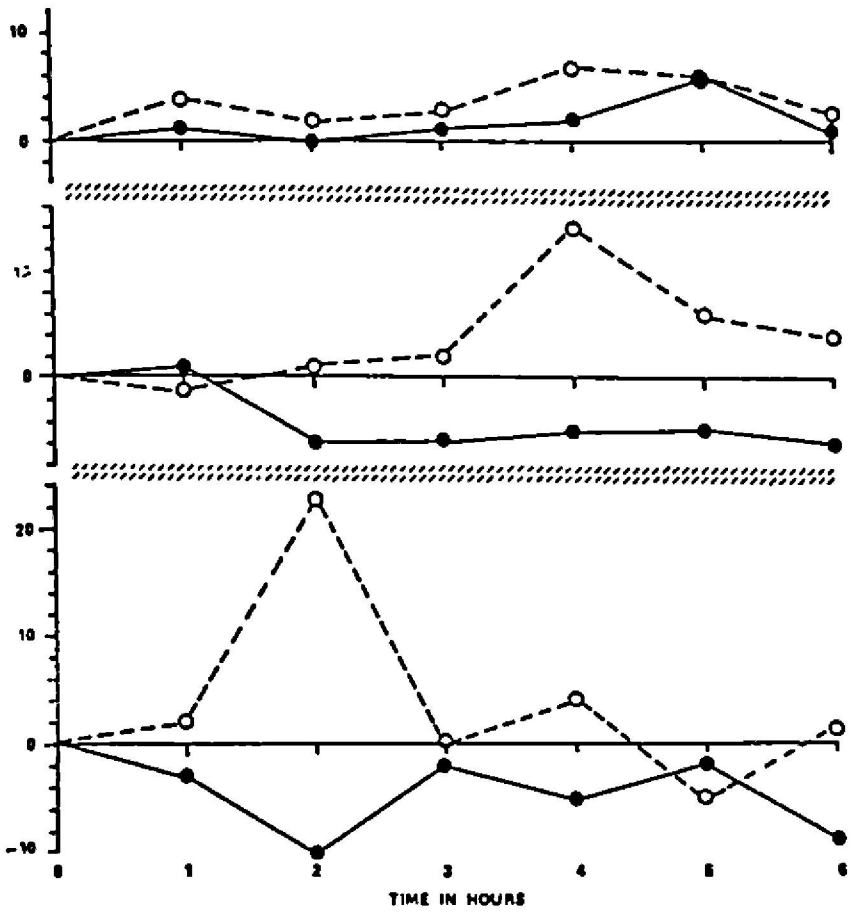

Fig. 5. Curves of change in breath H_2 concentration at 60-minute intervals over 6 hours in three lactose absorbers who ingested 360 ml cow's milk (containing 18 gm intrinsic lactose) either alone (-●-) or mixed with 18 gm polydextrose (-o-) in experiment 4. All subjects had flat curves with milk alone, but a range of individual breath H_2 responses to added polydextrose was seen.

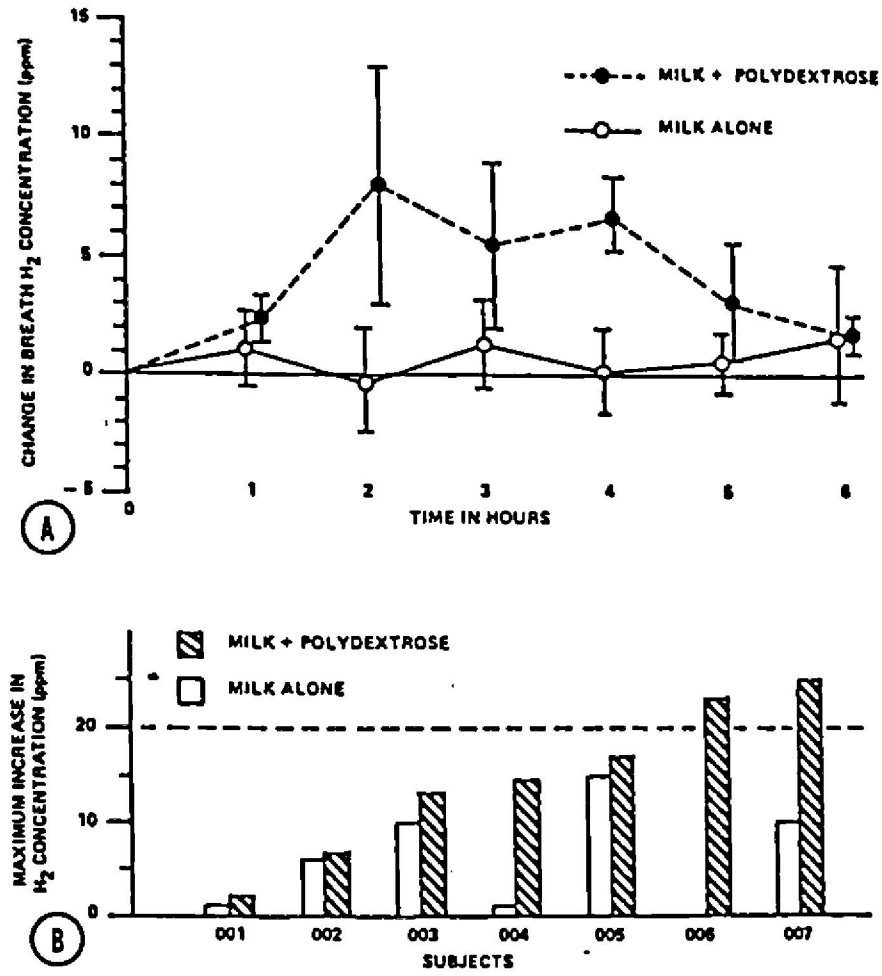

Fig. 6. A, Composite curves of mean change in breath H_2 concentration at 60-minute intervals over 6 hours in seven lactose-absorbers in experiment 4 who ingested 18 gm lactose in 360 ml cow's milk, alone (-●-) or with 18 gm polydextrose added (-o-). B, Individual maximum increments in breath H_2 concentration with two test beverages for each of seven subjects; two volunteers manifested ≥20 ppm rise in breath H_2 with polydextrose-added milk.

cretion.[21,22] The H_2 response behavior after oral administration of lactulose and glucose, therefore, established patterns for comparison for the in vivo response to our unknown carbohydrate polymer polydextrose. On the basis of the breath H_2 curves, polydextrose in water performed as did native glucose. We had to rule out a slower intracolonic metabolism of the polymer as the source of the flat H_2 response during the first 5 hours after ingestion. Oligosaccharides such as raffinose and stachyose show a delayed appearance of H_2 as compared with lactulose in the same individuals,[23] probably because of the more complex intracolonic hydrolyses required to liberate monosaccharide. Prolonged observation for up to 10 hours in three subjects, however, failed to reveal any increments of breath H_2 >15 ppm. We also had to address the obvious possibility that the flat H_2 response might have resulted from small bowel digestion of polydextrose and hence might represent complete intestinal absorption of the carbohydrate load. To clarify this issue, we used the conventional "glucose tolerance test" approach, that is, measuring the postdose increment in blood glucose. To assure detectable increments in glycemia, we used a larger dose of carbohydrate, 25 gm. Twenty-five grams of polydextrose also produced a flat blood glucose response, resolving any suspicion that its hydrolysis to elemental glucose and subsequent intestinal uptake was the cause of the failure to produce intestinal fermentation. As with the 15 gm aqueous doses, the accompanying breath H_2 response pattern was essentially flat with 25 gm polydextrose and identical to the response with 25 gm absorbable glucose.

The liberation of H_2 when substrates are incubated at 37° C with stool homogenates has been used as an index of fecal fermentation.[14,15,24-27] Gram for gram, all fermentable sugars yield an equivalent amount of H_2 into a closed incubation system.[14] Glucose is the parent compound for the polydextrose polymer, and it represented the logical standard for comparison in vitro. Moreover, inasmuch as exposure of the stools to atmospheric conditions before and during incubation has variable effects on the sensitive anaerobic flora that produce H_2,[27] the most valid approach to the incubation experiment was to use the mean of glucose fermentation results for each individual as the standard (100%) for reference to the H_2 production efficiency with polydextrose. In almost perfect agreement with the $^{14}CO_2$ studies,[5] which estimated a 25% overall fermentability of polydextrose, is our mean value of 24.8% fermentation of the polymer as compared with an equal amount of glucose.

Based on the 25% fermentation of polydextrose, and on the fact that the energy from fermentable sub-

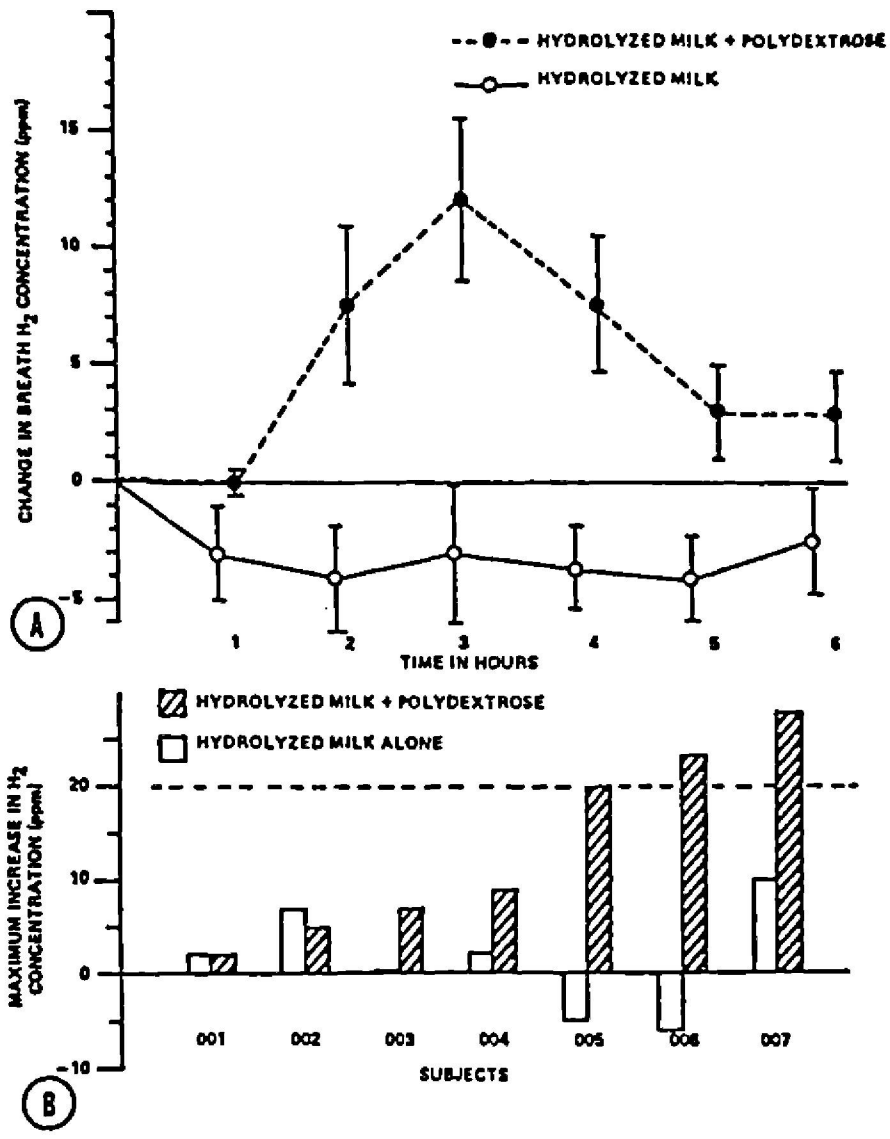

Fig. 7. A, Composite curves of mean change in breath H_2 concentration at 60-minute intervals over 6 hours in seven healthy volunteers in experiment 5 who ingested 360 ml cow's milk with lactose prehydrolyzed by prior incubation with a β-galactosidase, with (-c-) or without (-●-) addition of 18 gm polydextrose. B, Individual maximum increments in breath H_2 concentration with two test beverages for each of seven volunteers; three subjects manifested a ≥20 ppm rise in breath H_2 with polydextrose added to lactose-prehydrolyzed milk.

strates can be absorbed in a utilizable form from the colon,[28-32] Figdore and Bianchine[6] estimated that up to one fourth of the caloric value of polydextrose was available to human consumers. Of particular note is the wide dispersal of data from our in vitro study, from 7% to 45% relative efficiency. Translated into human terms, some individuals would be expected to obtain up to 1.8 kcal metabolizable energy from each gram of polydextrose ingested, whereas others would receive essentially none. Moreover, extrapolating to the in vivo situation, if fermentation took place slowly over time in the colon, the average of 3.75 gm (25% of the 15 gm polydextrose in water) might still have been fermented by the microflora without registering a detectable pulmonary H_2 response. Stephen et al.[33] recently showed by quantitative intestinal perfusion techniques that up to 6.3 gm starch can pass into the colon without registering any rise in breath H_2 concentrations over 6 hours of observation. Thus, there is no inherent conflict between the aqueous solution studies in vivo, even carried out to 10 hours, and the fecal incubation results. Assuming very slow metabolism of the 25% of the polydextrose that might have been fermented with the 15 gm aqueous dose, the data in Fig. 2 in vivo can be harmonized with the in vitro findings of Fig. 3.

The results obtained when polydextrose was mixed into a milk beverage, however, were intriguing and perplexing. In the aggregate, a slight but statistically significant increase in mean breath H_2 excretion was seen in the seven lactose-absorbing individuals who took 18 gm polydextrose with 18 gm lactose as milk. More important, in two of seven individuals a peak increment was manifested in breath H_2 concentration representing biologically significant carbohydrate fermentation: ≥20 ppm. Considering the possibility that this was caused by the inhibition of intestinal lactase by the polydextrose, we repeated the experiment with 18 gm polydextrose added to 260 gm lactose-prehydrolyzed milk, and found virtually the identical phenomenon, this time in three of seven volunteers a peak increment of breath H_2 of ≥20 ppm, despite the monosaccharide form of the milk carbohydrate.

We can only speculate as to the origins of this greater apparent colonic fermentation (breath H_2 excretion) when polydextrose is combined with a beverage. The first possibility is that polydextrose caused incomplete absorption of the companion carbohydrate(s), perhaps through an effect on intestinal transit; that is, that maldigested lactose, on the one hand, or malabsorbed glucose and galactose, on the other, is the source of the enhanced colonic fermentation. As an alternative, but far less likely, is the proposition that some component in the milk—carbohydrate or noncarbohydrate—interacts with the polydextrose, making the efficiency of the colonic fermentation of the polymer greater than that seen with the aqueous solutions in water alone. Our data do not allow any resolution of this issue. However, if we assume that whatever carbohydrate contributing to the fermentation process with polydextrose in the milk was handled identically to lactulose by the colonic flora, then a semiquantitative estimation of the amount of carbohydrate being metabolized could be made. This process of estimation of the degree of carbohydrate lost to the colon during breath tests was recently used with success in the studies of the autodigestion of yogurt lactose by Kolars et al.[34] Thus, relating the areas under the breath H_2 curves in Figs. 6 and 7 from experiments 4 and 5 with milk plus polydextrose to the breath H_2 response to aqueous lactulose in Fig. 2, we would estimate that the postdose production of H_2 represents colonic fermentation of 3 to 4 gm of the total 36 gm carbohydrate load in the test beverages. This represents 16 kcal dietary energy, some of which would likely be

recovered by the body in the process of colonic conservation.[28-32]

Polydextrose taken alone seems to fulfill the criteria for a useful bulking agent for dietary energy dilution, being nonabsorbable and poorly fermented. However, it is in the context of a meal that the polymer is meant to be used. With addition of 18 gm polydextrose to milk, a greater amount of colonic fermentation occurred than with the milk beverages alone. Three conditional points must be made, however. First, our dose of polydextrose, 18 gm, was larger than the 15 gm approved by the Food and Drug Administration for inclusion in a "serving" of a dessert or pastry, but with a "second helping" of a polydextrose-containing confection at a given meal, this 15 gm limit is immediately exceeded. Second, the mere presence of H_2 production does not correlate well with the symptoms of gastrointestinal intolerance.[35] It simply indicates the colonic arrival of appreciable amounts of fermentable substrate. The fact that we did have reports of mild intolerance symptoms with the milk and polydextrose beverages bears further examination and exploration in a properly designed, double-blind, and disguised protocol. Third, we provided polydextrose with only one food, milk. This was chosen because it is a popular accompaniment to pastries and dessert snacks. It will be important to pursue the investigation of whether more fermentation is manifest after consumption of other foods and beverages with polydextrose than without the polymer.

Physiologic studies can provide only predictive insights into the anticipated in vivo behavior. Our data suggest that further evaluation of the potential interaction of random-bonded glucose polymers with other food components be undertaken to define the basis of increased breath H_2 excretion when polydextrose is combined with milk. Ultimately, however, the safety and efficacy of a given strategy for weight reduction, such as dietary dilution with synthetic polymers, can be evaluated only by epidemiologic observations among consumers.

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ERRATUM

In the article entitled "Adrenaline and adenosine diphosphate-induced platelet aggregation require shape change Importance of pseudopods" by John G Milton and Mony M Frojmovic in the November issue (104 805-815, 1984), the Abstract and Abbreviations have errata.

In the Abstract, lines 5 and 6 should read. Citrated platelet-rich plasma prepared at 37° C contains ~65% discocytes and ~35% discoid platelets, which possess pseudopods, $(DE)_0$.

Lines 15 through 17 should read However, in both cases, platelet aggregation does not exceed the total number of shape-changed platelets (i e., $(DE)_0$ plus newly shape-changed discocytes), indicating that $(DE)_0$ are also participating in platelet aggregation.

The Abbreviations should include $(DE)_0$ = disco-echinocytes before addition of activator.