PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 80–85 (1982)

Intraintestinal Carbohydrate in Rats during the Feeding of Glucose or of Raw or Cooked Cornstarch[1] (41311)

M. A. MALIK,*[,2] D. L. TROUT,*[,3] B. C. EMAMALI,† AND D. J. BRENDZA†

*Carbohydrate Nutrition Laboratory, Beltsville Human Nutrition Research Center, Human Nutrition, Science and Education Administration, U.S. Department of Agriculture, Beltsville, Maryland 20705; and †Department of Food, Nutrition, and Institution Administration, University of Maryland, College Park, Maryland 20742

Abstract. The quantity of free and total (measured after hydrolysis) glucose in the small intestine was examined in rats during a daily period of high food intake. In experiment 1, rats were fed a high-glucose diet, and intestinal contents for analysis were collected by several procedures, all of which yielded similar results. In experiment 2, nutritionally adequate diets containing 66.9% carbohydrate as glucose or cooked (pregelatinized) or raw cornstarch were fed *ad libitum* for 13 days. The small intestine then contained 37 and 40.6 mg free and total glucose, respectively, in glucose-fed rats, similar amounts of both components in animals fed the cooked starch, and 600+% more total glucose in rats fed raw cornstarch than glucose. In all three dietary groups, total glucose content of the intestine was positively correlated with dry matter content of the stomach.

The feeding of some but not all starches in place of glucose in nutritionally adequate diets reduces total liver lipids in rats and also the activities of some enzymes related to lipogenesis (1–4). The reason for different responses is not altogether clear. Some starches do affect energy intake by animals and differ in the completeness of their digestion in the intestinal tract (5, 6). We suggest that the degree of delay in the hydrolysis of different starches in the small intestine could affect both the amount and distribution of carbohydrate along the intestine and could influence variability in the rate of intestinal absorption of glucose and that these gastrointestinal effects might be responsible for nutritional effects.

In an attempt to examine the hypothesis, rats were *ad libitum* fed carbohydrate diets containing either free glucose or a starch which was easy or difficult to hydrolyze; and we have examined the amount of carbohydrate in the small intestine of these animals during a period of high food intake. It was recognized that the intraintestinal carbohydrate, particularly in glucose-fed animals, represents a small and labile pool which could change markedly at the time of death if gastric emptying and intestinal absorption were then disturbed to different degrees. In experiment 1, we tested the effect of several methods of killing on the amount of carbohydrate recovered from the small intestine of glucose-fed rats. In experiment 2, we fed diets containing 66.9% raw cornstarch, cooked (pregelatinized) cornstarch, or anhydrous glucose and determined the amounts of free and total (i.e., measured after enzymatic hydrolysis) glucose in their intestine.

Materials and Methods. *Animals.* Male Wistar rats, weighing 125–180 g, were obtained from Hilltop Laboratory Animals, Inc., Scottdale, Pennsylvania. Animals were immediately put on a 12:12-hr light cycle, with darkness starting each day at 0930 hr. Rats were *ad libitum* fed, for at least 13 days, one of the three diets described in Table I. Food intake was measured during the 3.7- to 7.9-hr period of the final dark cycle until intestines were removed.

[1] Mention of a trademark or proprietary product 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 that may also be suitable.

[2] Present address: Epidemiology Branch, Food Safety and Quality Service, U.S. Department of Agriculture, Beltsville, Md. 20705.

[3] To whom requests for reprints should be addressed.

0037-9727/82/010080-06$01.00/0

TABLE I. COMPOSITION OF DIETS

	Percentage
Carbohydrate[a]	66.91
Lactalbumin[b]	10.00
Casein, vitamin free[b]	10.00
Partially hydrogenated vegetable oil (Crisco)	3.00
Corn oil (Mazola)	2.00
Vitamin mix, AOAC (7)[c]	1.00
Mineral mix, AIN-76 (8)[c,d]	3.09
Nonnutritive cellulose (Alphacel)[b]	4.00

[a] Either anhydrous glucose (Teklad), cooked (pregelatinized) cornstarch (National), or raw cornstarch (Teklad).
[b] From ICN Pharmaceutical Company.
[c] From Teklad Diets.
[d] Specially compounded without carbohydrate.

Experiment 1. The high-glucose diet of Table I was fed to 28 rats, and 14 of these were fitted with a device for occluding the pyloric sphincter immediately after decapitation. The latter animals were first anesthetized with sodium secobarbital, and their abdominal cavity was opened. A flexible tube (PE-60, intramedic, nonradiopaque polyethylene tubing, Clay Adams) was passed throught the body wall, under the skin and out between the scapulae. A silk thread was looped loosely around the pyloric sphincter, and both ends were passed through the tube and knotted just outside the body. The incision was then closed. Animals were allowed 7–13 days to recover. All were gaining weight when killed.

The small intestine and stomach were removed promptly after one of the following four procedures: (1) surgically prepared rats were guillotined, and the pyloric sphincter was immediately occluded by a pull on the implanted loop; (2) surgically prepared animals were guillotined, but the loop was not pulled; (3) intact animals were guillotined; and (4) intact animals were not guillotined but were anesthetized with sodium amobarbital (100 mg/kg ip). The body cavity was first opened. The stomach was clamped just above the pyloric sphincter. While the proximal duodenum was compressed, the pyloric sphincter was severed; and the small intestine was carefully pulled free of mesentery. The intestine was transected just above the caecum, placed in 10% trichloroacetic acid (TCA), and homogenized in a Virtus homogenizer. The interval from decapitation to intestinal submergence in TCA averaged 100 sec. Stomachs were tied at both the esophageal and pyloric ends, removed, and stored frozen for up to 2 weeks.

Samples of the intestinal homogenate were filtered, heat treated in 1.1 *N* hydrochloric acid (9), neutralized and analyzed for glucose by the method of Dubowski (10). Stomach contents were transferred quantitatively to weighed beakers, as previously described (11), diluted and analyzed for glucose (10).

Experiment 2. Groups of eight rats were each *ad libitum* fed, for 13 days, one of the three diets from Table I. They were weighed daily between 0830 and 0930 hr. Food intake was measured for 48 hr prior to the final daily weighing.

The procedure for removing the small intestine was modified as a result of findings from experiment 1, and these changes are described under Results. The intact intestine was promptly placed in a cold fluoride-containing, sodium citrate buffer (pH 4.2) (12) designed to inhibit glycolysis and the hydrolysis of starch by pancreatic amylase; and was promptly homogenized. The homogenization flask and contents were placed for 5 min in a boiling water bath. This treatment inactivated intestinal enzymes and gelatinized the starch, when present. An aliquot of the homogenate was filtered and analyzed enzymatically for free glucose (13). The remainder was incubated overnight at room temperature with 1 mg *Rhizopus* amyloglucosidase (from Sigma Chemical Corp.) per milliliter sample. An aliquot of the sample was filtered and was also analyzed enzymatically for glucose, which, in this case, represented both initially free and diomerized and polymerized glucose. This was called "total glucose."

The enzymatic procedure for glucose measured spectrophotometrically the NADPH formed by the coupled hexokinase and glucose-6-phosphate dehydrogenase reactions (13). A kit of reagents and enzymes from Calbiochem–Behring Corpo-

ration was used. A 3.0-ml portion of the reagent mixture obtained by diluting and mixing reagents provided sufficient buffer capacity to accommodate at least 0.04 ml of the mildly acid intestinal extracts.

Stomachs were also removed and promptly frozen. Within 2 weeks, their contents were removed (11), dried overnight at 100°, and weighed.

Results. *Experiment 1.* None of the terminal procedures affected significantly the amount of carbohydrate recovered in the small intestine. There was no clear indication of a large postmortem discharge of stomach contents through the pyloric sphincter in any of the rats (Table II). It was decided, for experiment 2, that pyloric loops would not be installed and that animals would be killed by decapitation. The time for removing the intestines was reduced usually to less than 75 sec.

The rats of experiment 1 were apparently caught at greatly different stages between meals, for the glucose contents of the stomachs ranged between 374 and 10,600 mg/kg body weight. It was decided, for experiment 2, that animals would not be killed until 4.3 hr into the dark cycle and then only when they had eaten at least 2 g diet since the previous light period. Two animals per group were killed on each of 4 days.

Experiment 2. The procedure for determining total glucose in intestinal contents was tested against weighed samples of raw cornstarch and yielded 95+% of the amount of glucose theoretically present. Comparisons of values for free and total glucose in the small intestine, as reported in Table III, provided further tests of the analytical procedure. In glucose-fed rats, total glucose exceeded the 37.0 mg value for free glucose by 3.6 mg or 10%. The extra glucose released by amyloglucosidase is thought to have been derived from glycogen and some glycoprotein in the intestinal wall. The difference between free and total glucose exceeded 10% in starch-fed animals and is thought, in this case, largely to represent incompletely hydrolyzed starch.

Weight gains of rats in the three diet groups averaged 5.5–6.7 g per day for the 13-day period when test diets of Table I were fed. Animals fed the diet containing cooked cornstarch, however, gained slightly but significantly less than did those fed either of the other two diets.

The amount of intestinal free and total glucose did not greatly differ between rats fed either the glucose or cooked cornstarch diet. For animals fed the cooked cornstarch, incompletely hydrolyzed starch and glycogen represented only 28% of total glucose. For animals fed the raw cornstarch diet, free and total glucose content of the intestine was, respectively, 77% and 600+% higher than for glucose-fed rats.

Discussion. We have observed (unpublished data), while *ad libitum* feeding a

TABLE II. CARBOHYDRATE RECOVERY FROM STOMACH AND SMALL INTESTINE OF GLUCOSE-FED RATS AFTER VARIOUS TERMINAL PROCEDURES (EXPERIMENT 1)

Terminal procedure	Final body weight (g)	Time of kill[a] (hr)	mg/kg body weight	
			Glucose in stomach[b]	Carbohydrate in small intestine[b]
Decapitated; loop pulled	275 (237–358)[d]	4.5 (3.2–6.0)[d]	2150 ± 600[c]	78 ± 5
Decapitated; loop not pulled	296 (237–371)	5.0 (3.7–5.6)	3060 ± 930	108 ± 15
Intact; decapitated	277 (212–336)	5.4 (4.3–7.9)	4350 ± 1530	94 ± 16
Intact; anesthetized	271 (187–346)	5.7 (3.7–7.3)	3640 ± 750	87 ± 13

[a] Measured from start of dark period at 0930 hr.
[b] "Carbohydrate" is the amount of glucose recovered in an aqueous extract after hydrolysis in 1.1 *N* HCl.
[c] Mean ± SEM for groups of seven rats.
[d] Numbers in parentheses indicate range.

TABLE III. DIETARY CONDITION OF RATS AND AMOUNTS OF FREE AND TOTAL GLUCOSE RECOVERED FROM THEIR SMALL INTESTINES (EXPERIMENT 2)

	Raw cornstarch diet	Cooked cornstarch diet	Glucose diet
(1) Days of *ad libitum* feeding	13	13	13
(2) Body weight gains, g[a,b]	85.9 ± 4.5[1]	71.8 ± 4.6[2]	87.6 ± 3.1[1]
(3) Final body weights, g	308 ± 5[1]	294 ± 5[2]	310 ± 4[1]
(4) Food intake for final 2 days, g[c]	45.9 ± 1.0	44.0 ± 1.9	45.5 ± 1.5
(5) Time of kill from start of dark, hr	5.5	5.5	5.5
Range	(4.3–6.7)	(4.5–6.8)	(4.8–7.0)
(6) Amount eaten during final dark period, g	8.53 ± 0.85	7.68 ± 1.31	6.96 ± 0.71
(7) Dry matter in stomach, mg	1340 ± 260	1270 ± 270	1240 ± 330
In small intestine,			
(8) Free glucose, mg	65.6 ± 8.2[1]	38.7 ± 5.2[2]	37.0 ± 10.1[2]
(9) Total glucose, mg[d]	290.0 ± 40.2[1]	53.4 ± 4.3[2]	40.6 ± 10.8[2]
(10) Correlation coefficient between (7) and (9)	0.72[e]	0.85[e]	0.76[e]

[a] Mean ± SEM with eight rats per group.
[b] Means having different superscript numbers are different ($P < 0.05$) by Duncan's multiple range test.
[c] Measured during 48-hr period ending at 0900–0930 hr on final day.
[d] Amount of glucose recovered after hydrolysis of sample with fungal amyloglucosidase.
[e] Significantly different ($P < 0.05$) from zero.

high-glucose diet that was almost identical to the present one, that Wistar rats consumed 30% of their daily intake between 4 and 8 hr after the start of the 12-hr period of darkness. Gastric emptying of glucose then proceeded at an average rate of 2.2 g/hr/kg metabolic body weight, i.e., per (kg body weight)$^{3/4}$. In experiment 2, the average body weight of 310 g for animals fed the glucose diet represents 0.415 kg metabolic body weight. For rats of this size, the previously observed gastric emptying rate corresponds to 15.1 mg glucose/min. The 37 mg free glucose recovered in the small intestine of glucose-fed animals (Table III) corresponds to an amount discharged by the stomach at its average rate for 2.5 min. This finding implies relatively prompt absorption of glucose in the intestine, and it reinforces the notion that for simple sugars, particularly, gastric emptying controls the rate of intestinal absorption (14).

Undoubtedly, hydrolysis of starch by pancreatic amylase persisted after decapitation during the 75 sec required for the removal of the intestine. Postmortem hydrolysis appears to cause an overestimation of free glucose in the intestines of starch-fed animals. Despite some quantitative uncertainty, the fraction of the intestinal carbohydrate that had been hydrolyzed to glucose was far higher, 72 versus 23%, when cooked rather than raw cornstarch was fed.

The main finding of the present study was that the small intestine of rats fed the raw cornstarch diet contained 400+ and 600+% more total glucose than that from rats fed cooked cornstarch or free glucose, respectively. The extra carbohydrate recovered with the feeding of raw cornstarch appears to reflect slow hydrolysis of the starch in the intestine and the persistence of glucose polymers that were too large for absorption. Pregelatinization of the starch enhanced its susceptibility to amylase activity and reduced by 90% the amount of glucose complexes remaining in the intestine.

The question arises how a delayed hydrolysis of starch might act to depress hepatic glucose-6-phosphate dehydrogenase and malic enzyme. It has been reported that, during "meal-training" to a single 2-hr eating period per day (15, 16) and also during the refeeding of starved rats (17, 18), an increase occurs first in lipogenesis and then in activities of some lipogenic enzymes.

Apparently, lipogenesis and enzyme activities are affected by periods of high substrate availability. We propose that the susceptibility of starches to hydrolysis in the intestine may affect peak rates of hepatic uptake of glucose by influencing digestive and endocrine processes.

Simple sugars are, in effect, introduced into the intestine at a more distal region when the fed starch undergoes slow rather than rapid hydrolysis. Gastroinhibitory polypeptide (GIP), which is physiologically important in potentiating insulin release by circulating glucose in the pancreas, is unevenly distributed in the intestinal tract (19). In man, an infusion of glucose elevates serum GIP more markedly when it is introduced into the proximal portion, rather than into a more distal region, of the intestine (20). Some other enteric hormones, whose secretion may or may not be affected by ingested glucose, are also unevenly distributed within the intestinal tract (21). The amount and distribution of glucose within the intestinal tract, which is influenced by the kind of starch fed, may have important hormonal implications which affect hepatic lipogenesis.

The delivery of carbohydrate from the stomach to the intestine and blood also warrants comment. The stomach delivers nutrient energy from various nutrient sources to the intestine at relatively controlled rates (22). During eating, however, a bolus of unregulated energy content enters the duodenum (23). Prior to the next meal, gastric emptying may slow down, as gastric contents are depleted and becomes increasingly acid (14). The correlation that we observed between gastric dry matter content and intestinal carbohydrate lends further support to the concept that during periods of high food intake, the rate of entry of carbohydrate into the intestine fluctuates between meals.

In experiment 2, raw cornstarch was well tolerated and supported a high rate of growth. During the middle of the dark period, the total glucose recovered in the intestine of rats fed the raw starch approximated the amount of estimated delivery from the stomach in a 19-min period. Meanwhile, rats were eating definite meals at intervals of perhaps 1–3 hr (24). We think that, when raw cornstarch was fed, its relatively slow hydrolysis in the intestine reduced the rate of glucose absorption during meals, sustained glucose absorption as emptying slowed prior to the next meal, and tended to dampen out fluctuations in the rate of delivery of nutrient energy to the metabolic system during periods of rapid food intake.

Our findings indicate that (i) the amount of free and total glucose in the small intestine can be satisfactorily measured; (ii) the amount of total glucose in the small intestine may be greatly increased by the feeding of a moderately amylase-resistant, but still well-utilized, starch; and (iii) the measurement of free and total glucose helps clarify physiologically important events occurring in the intestinal tract.

1. Michaelis IV OE, Nace CS, Szepesi B. Brit J Nutr **39**:85, 1978.
2. Michaelis IV OE, Hallfrisch JG, Putney JD, Scholfield DJ, Reiser S. J Nutr **107**:2171, 1977.
3. Chang MLW, Lee JA, Schuster EM, Trout DL. J Nutr **101**:323, 1971.
4. Naismith DJ, Rana IA. Nutr Metab **16**:285, 1974.
5. Booher LE, Behan I, McMeans E. J Nutr **45**:75, 1951.
6. Reussner G Jr, Andros J, Thiessen R Jr. J Nutr **80**:291, 1963.
7. Association of Official Agricultural Chemists. In: Official Methods of Analysis. Washington, D.C., Assoc. Offic. Agr. Chem., p680, 1960.
8. Bieri JG, Stoewsand GS, Briggs GM, Phillips RW, Woodard JC, Knapka JJ. J Nutr **107**:1340, 1977.
9. Association of Official Agricultural Chemists. In: Official Methods of Analysis. Washington, D.C., Assoc. Offic. Agr. Chem., p374, 1955.
10. Dubowski KM. Clin Chem **8**:215, 1962.
11. Trout DL, Conway ES, Putney JD. J Nutr **107**:104, 1977
12. Murat CC, Serfaty A. Clin Chem **20**:1576, 1974.
13. Slein MW. In: Bergmeyer H-U, ed. Methods of Enzymatic Analysis. New York, Academic Press, p117, 1963.
14. Hunt JN, Knox MT. In: Handbook of Physiology. Washington, D.C., Amer. Physiol. Soc., Sect. 6, Vol. IV: p1917, 1968.
15. Armstrong MK, Romsos DR, Leveille GA. J Nutr **106**:884, 1976.

16. Leveille GA. J Nutr **90**:449, 1966.
17. Tepperman J, Tepperman HM, Amer J Physiol **200**:1069, 1961.
18. Foster DW, Srere PA. J Biol Chem **243**:1926, 1968.
19. Brown JC, Frost JL, Kwauk S, Otte SC, McIntosh CHS. In: Glass GBJ, ed. Gastrointestinal Hormones. New York, Raven Press, p223, 1980.
20. Thomas FB, Shook DF, O'Dorisio TM, Cataland S, Mekhjian HS, Caldwell JH, Mazzaferri EL. Gastroenterology **72**:49, 1977.
21. Solcia E, Capella C, Buffa R, Frigerio B, Usellini L, Fiocca R. In: Glass GBJ, ed. Gastrointestinal Hormones. New York, Raven Press, p1, 1980.
22. Hunt JN, Stubbs DF. J Physiol (London) **245**:209, 1975.
23. McHugh PR, Moran TH. Amer J Physiol **236**: R254, 1979.
24. Balagura S, Devenport LD. J Comp Physiol Psych **71**:357, 1970.

Received May 26, 1981. P.S.E.B.M. 1982, Vol. 169.