Small Intestinal Glucose Transport

Proximal-Distal kinetic gradients

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ABSTRACT Proximal and distal small intestinal segments of the rat were perfused *in situ* at two different rates with isotonic solutions containing glucose in concentrations ranging from 25 to 600 mg/100 ml. Absorption was measured as glucose disappearance rate from the lumen. Glucose absorption had not previously been studied at intraluminal concentrations above and below blood glucose. Absorption was more rapid from the proximal segment. In both segments absorption was independent of perfusion rate and of whether glucose was analyzed by counting ^{14}C or by the Somogyi method. The latter finding suggests that of the unidirectional fluxes, flux out of the bowel is much greater than flux into the bowel. In contrast to the findings in previous studies neither segment showed rate-limiting kinetics, and the Michaelis-Menten analysis was not applicable. The form of the curve depicting absorption rate in relation to concentration differed between the two segments. At the higher concentrations absorption rate continued to increase much more rapidly in the proximal than in the distal segment. The observations could not be explained by known mechanisms of glucose transport and illustrate the difficulties of achieving biochemically and physiologically meaningful in vivo studies of intestinal absorption.

Previous investigations of the kinetics of glucose transport have shown differences among various organs and tissues. Studies reported here involved in situ perfusion of the rat small intestine and demonstrate differences in transport kinetics in proximal and distal portions of the same organ.

METHODS AND MATERIALS

Male albino rats of the Sprague-Dawley strain weighing 200–300 g were fasted for 20 hr. After anesthesia with intraperitoneal sodium pentobarbital (20 to 40 mg/kg), the abdomen was opened by a midline incision. The pylorus, common bile duct, and

terminal ileum were ligated. The intestine was divided into two parts with the blood supply intact; a proximal segment measuring 30–50 cm starting from the pylorus, and a distal segment of similar length, just proximal to the cecum. An inlet tube was tied through a small incision into the proximal end, and an exit tube into the distal end of each segment. Buffered isotonic glucose solutions were pumped into the proximal end of each segment with a constant infusion pump (Bowman Pump, Process and Instruments, Brooklyn, N. Y.). Two rates were used: 0.5 and 1.35 ml/min. After a 45 min wash period, three 15 min samples were collected concurrently from each segment. At the end of the experiment the segments were removed and measured. Absorption was expressed as rate per 10 cm of intestine.

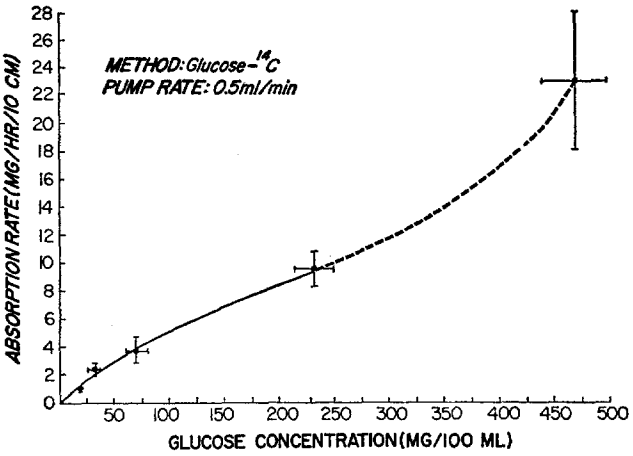

FIGURE 1. Proximal small intestinal glucose absorption (glucose-^{14}C) in relation to geometric mean concentration at the slow pump rate (0.5 ml/min). Means and one standard deviation of rates and geometric mean concentrations are plotted. At lower concentrations the curve suggests rate-limiting kinetics (solid line), but become S-shaped as the concentration is raised (dashed line).

The solutions contained glucose in concentrations of 25, 50, 100, 300, and 600 mg/100 ml in a modified Ringer solution (9). The solutions were maintained isosmotic by adjusting the NaCl content. Phenol red (PR), (20 mg/liter), was added to each solution as the unabsorbed indicator (9). A tracer amount (0.02 mc, 5.22 μmole, 0.94 mg) of ^{14}C-labeled glucose was added to each liter of solution.

Absorption is defined as disappearance of glucose from the gut lumen, measured as the difference between initial glucose concentration in the solution perfused and final concentration in the samples. Glucose concentration was analyzed by the Somogyi-Nelson method (12) and by liquid scintillation counting of ^{14}C-labeled glucose. The counting vials contained 0.2 ml of sample, 2.0 ml of water, 2 drops 12.5 N NaOH, and 10 ml of scintillation solvent (3). The alkaline solution decreased quenching by phenol red and gave counting efficiencies of about 50%. The samples were counted in a Packard Tri-Carb scintillation spectrometer model 3003. PR was analyzed in a Beckman DU spectrophotometer at 520, 560, and 600 mμ as previously

described (10) using 1.0 ml of the perfusion solution and samples, 5.0 ml of water, and 1.0 ml of pH 9.2 borate buffer. In the following formulas G refers to glucose, the subscripts I and F to initial and final concentrations, and IR to infusion rate.

$$G \text{ unabsorbed, } \% = 100\ (G_F/G_I)\ (PR_I/PR_F)$$

$$G \text{ absorbed, } \% = 100\% - G \text{ unabsorbed, } \%$$

$$G \text{ absorbed mg/hr/10 cm} = \frac{(G_I, \text{mg/ml})(IR, \text{ml/hr})\ G \text{ absorbed, } \%}{(100)(\text{gut length, cm}/10)}$$

TABLE I
PROXIMAL GLUCOSE ABSORPTION

	Slow pump rate										Fast pump rate				
	^{14}C data					Somogyi data					^{14}C data				
c_I*	c_{GM}‡	SD§	Abs.‖	SD§	n¶	c_{GM}‡	SD§	Abs.‖	SD§	n¶	c_{GM}‡	SD§	Abs.‖	SD§	n¶
25	18.5	1.6	1.08	0.24	11	18.2	2.0	1.01	0.22	11	22.1	1.0	1.13	0.31	12
50	32.3	3.9	2.31	0.48	7	35.4	3.7	1.97	0.42	7	43.8	2.0	2.34	0.74	11
100	68.6	9.4	3.67	0.80	6	71.2	7.7	3.46	0.77	6	88.3	3.3	4.04	1.08	15
300	231.8	17.7	9.49	1.24	10	240.2	18.8	8.37	1.74	10	270.4	9.6	10.63	4.28	6
600	469.4	28.7	22.95	5.04	8	466.2	43.9	22.76	6.98	8	—	—	—	—	—

* Initial concentration, mg/100 ml.
‡ Geometric mean concentration.
§ Standard deviation
‖ Absorption, mg/hr/10 cm.
¶ No. of rats.

$$\text{Geometric mean } G \text{ concentration } (C_{GM}) = \sqrt{G_I G_F}$$

$$\text{Standard deviation} = \sqrt{\frac{\Sigma X^2 - (\Sigma X)^2/n}{n-1}}$$

The reciprocals of the mean absorption rates were plotted against the reciprocals of the geometric mean concentrations (6), and a straight line was fitted to the points by the method of least squares. Constants analogous to K_M and V_{max} of enzyme kinetics were calculated from the slope and intercept of this line.

Calculations:

$$x = 1/\text{mean } C_{GM}$$

$$y = 1/\text{mean } G \text{ absorbed, mg/hr/10 cm}$$

$$n = \text{No. of concentration groups}$$

$$b = \{(\Sigma xy) - (\Sigma x \Sigma y/n)\}/\{(\Sigma x^2) - (\{\Sigma x\}^2/n)\} = \text{slope of curve of reciprocal plot}$$

$$a = (\Sigma y - b\Sigma x)/n = \text{intercept of slope on ordinate}$$

$$V_{max} = 1/a$$

$$K_M = b(V_{max})$$

RESULTS

Proximal small intestinal glucose absorption, measured using glucose-^{14}C, is shown in Fig. 1 and Table I. Initial glucose concentration was varied from 25 to 600 mg/100 ml. Absorption increases with concentration giving an S-shaped curve. The region of the curve above which an acceleration in absorption appears to be present is shown with the dashed line.

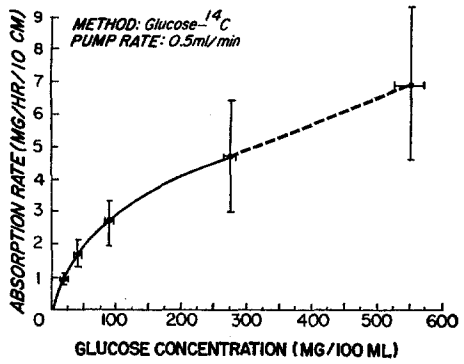

FIGURE 2. Distal small intestinal glucose absorption (glucose-^{14}C) in relation to geometric mean concentration at the slow pump rate. Means and one standard deviation of rates and geometric mean concentrations are given. At lower concentrations the curve rises more steeply, but tends to flatten at about 100 mg/100 ml. It does not show the acceleration that was demonstrated by the proximal segment as concentration was further increased. The dashed portion corresponds to the region of acceleration in the proximal segment.

Distal absorption data obtained at the same time and from solutions of the same concentration as the proximal are shown in Fig. 2 and Table II. The curve increases more steeply up to 100 mg/100 ml and then tends to flatten. The dashed portion of the curve corresponds to the region of accelerated absorption in Fig. 1.

Fig. 3 shows absorption in the proximal segment, measured by glucose-^{14}C, comparing the fast and slow pump rates. The data are nearly identical and indicate that absorption was independent of the rate of presentation of glucose load to the bowel at this level of glucose loading.

Proximal absorption data at the slow pump rate are nearly identical whether the samples are analyzed by counting glucose-^{14}C or by the Somogyi-Nelson method (Fig. 4 and Table I).

Table II compares distal glucose absorption data. Absorption data meas-

TABLE II
DISTAL GLUCOSE ABSORPTION

	Slow pump rate										Fast pump rate				
	^{14}C data					Somogyi data					^{14}C data				
c_I*	c_{GM}‡	SD§	Abs.‖	SD§	n¶	c_{GM}‡	SD§	Abs.‖	SD§	n¶	c_{GM}‡	SD§	Abs.‖	SD§	n¶
25	18.7	2.1	0.90	0.16	13	19.2	1.9	0.86	0.22	11	22.4	1.6	1.10	0.31	13
50	39.5	4.1	1.69	0.41	7	40.4	2.2	1.54	0.26	6	45.8	2.5	1.73	0.78	12
100	86.7	2.7	2.61	0.68	6	88.8	4.1	2.22	0.88	6	91.2	4.5	3.29	0.95	11
300	273.5	10.8	4.73	1.67	9	278.6	12.5	3.94	2.20	9	287.2	3.3	5.02	1.66	3
600	548.3	23.4	6.92	2.31	8	547.5	42.4	6.86	5.37	8					

* Initial concentration, mg/100 ml.
‡ Geometric mean concentration.
§ Standard deviation.
‖ Absorption, mg/hr/10 cm.
¶ No. of rats.

ured by glucose-^{14}C at the fast and slow pump rate and by the Somogyi method at the slow pump rate are in good agreement.

Kinetic constants derived from the ^{14}C data of these experiments are summarized in Table III. The highest glucose concentration used for calculation was 300 mg/100 ml. The concentration range corresponds to the solid portions of the curves in Figs. 1 and 2, and the dotted 1.35 ml/min curve in Fig. 3. The distal constants of the fast pump rates were calculated from the data

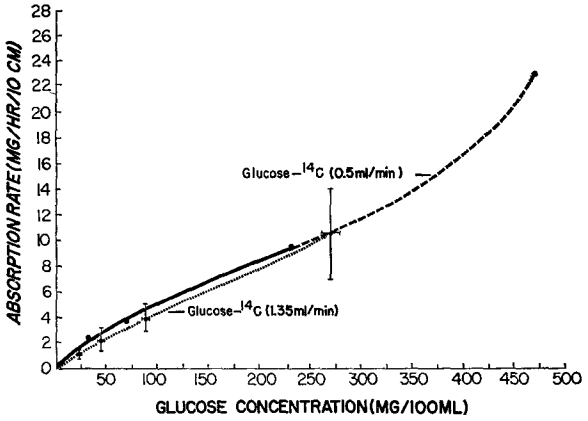

FIGURE 3. Proximal small intestinal glucose absorption (glucose-^{14}C) in relation to concentration at two pump rates. Means of rates and geometric mean concentrations are shown for both pump rates, and the standard deviations are given for the fast pump rate. Absorption is independent of pump rate but is a function of concentration.

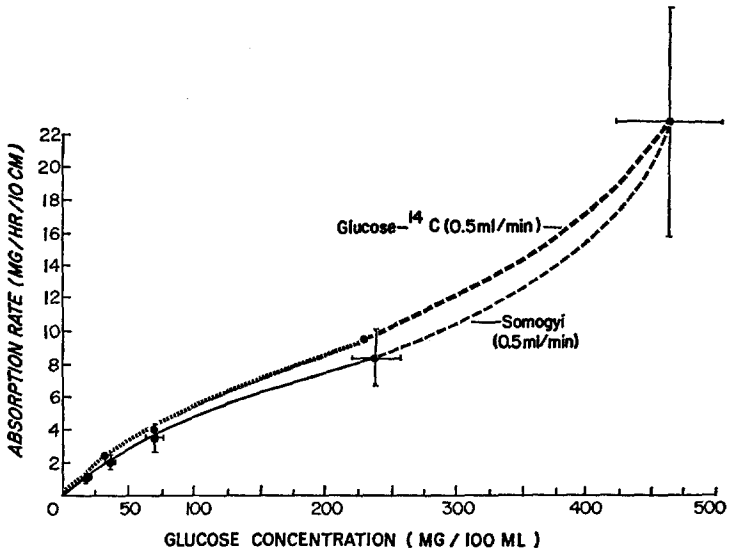

FIGURE 4. Proximal small intestinal glucose absorption. Comparison of glucose-^{14}C and Somogyi data at the slow pump rate. The same S-shaped curve was obtained by both analytical methods. Means and standard deviations are for Somogyi data and mean data only are shown for glucose-^{14}C (see Fig. 1).

in Table II. Proximal K_M and V_{max} values were eight times greater than the corresponding distal constants.

DISCUSSION

Glucose absorption rate in relation to its intraluminal concentration was studied in the proximal and distal halves of the rat small intestine. Rate-limiting kinetics were not observed in either segment. In the proximal segment (Fig. 1) the rate curve had steeper slopes at the lowest and highest concentrations with flattening between. The slight upward convexity of the curve over the 25-300 mg/100 ml initial concentration range was also observed at

TABLE III
KINETIC DATA*

Segment	Pump rate	K_M	V_{max}
	ml/min	*mM/liter*	*mg/hr/10 cm*
Proximal	0.5	47.4	47.6
	1.35	48.7	46.4
Distal	0.5	6.3	6.4
	1.35	6.9	7.1

* Based on absorption rates at initial concentrations from 25 to 300 mg/100 ml.
Glucose analysis was done by counting glucose-^{14}C.

the faster pump rate (Fig. 3) and was independent of whether glucose was analyzed by counting ^{14}C or by the Somogyi method (Fig. 4 and Table I). This portion of the curve approximates a straight line. The absorption rate at the initial glucose concentration of 600 mg/100 ml was measured at the slow pump rate, where the difference between initial and final concentration was sufficiently great to permit reliable estimation of absorption rate. There was no tendency for the absorptive process to show rate-limiting kinetics in the proximal segment. In fact the rate was somewhat above a straight line fitted through the data at the other concentrations. In the distal segment (Fig. 2) although the slope had flattened in comparison with the steep initial rise, the rate curve was still rising at the highest concentration studied. The data show a trend toward rate-limiting kinetics not at all evident in the proximal segment. That these results were probably valid was suggested by finding the same rate curve patterns for the proximal and distal segments regardless of whether glucose was analyzed by counting ^{14}C or by the Somogyi method (Fig. 4, Tables I and II). Absorption rates by the Somogyi method were consistently slightly lower, but both analytical methods gave essentially the same findings.

Although the usual analysis of absorption data by the Michaelis-Menten treatment (7) is not applicable to the present study, by using a limited concentration range a useful comparison with previous work is obtained. Thus, a K_M and V_{max} can be calculated for both segments using data up to C_I 300 mg/100 ml, since this portion of the rate curve is approximately a rectangular hyperbola (Table III). The results are depicted in relation to the rate curves in Fig. 5. The curves of both segments show a similar initial slope. They differ chiefly in the tendency for the distal curve to flatten and the proximal curve to continue its upward slope. The proximal K_M and V_{max} values lie outside the experimental portion of the curve. Absorption by the distal segment appears to fit Michaelis-Menten kinetics, but this is fortuitous as absorption was not rate-limited (Fig. 2).

The previous study of the kinetics of glucose absorption by the rat small intestine was in vitro (5). The data showed rate-limiting kinetics and were analyzed by the Michaelis-Menten treatment. Unlike the present study, the K_M values did not differ between the two segments. The K_M in vitro was about one-fifth that found for the proximal segment in the present in vivo study. Previous in vivo kinetic studies compared glucose transport by the proximal and distal small intestine in man (11) and the dog (1). In both species the values for K_M and V_{max} were greater in the proximal segment, just as in the present study. The differences between proximal and distal K_M values were similar to the findings of the present study. In man the proximal K_M was 73 and the distal 31 mM/liter. In the dog proximal K_M values ranged from 60 to 109 and the distal from 6 to 32 mM/liter. The greater V_{max} corresponds to the higher absorption rate usually observed in proximal segments both in vitro

(2, 5) and in vivo (4). The in vivo absorption curves in man and the dog resembled those of the present study in that the proximal absorption rates continued to increase and the distal rate curves tended to flatten at the highest concentrations.

In the previous in vivo studies cited above absorption was measured only under conditions where the luminal glucose concentration was greater than that of blood glucose. In the present study blood glucose was in the range of 100 to 150 mg/100 ml. Thus, the first three points of the curves (Figs. 1 and 2) presumably represent absorption against a concentration gradient. At the two highest concentrations downhill glucose transport was also occurring.

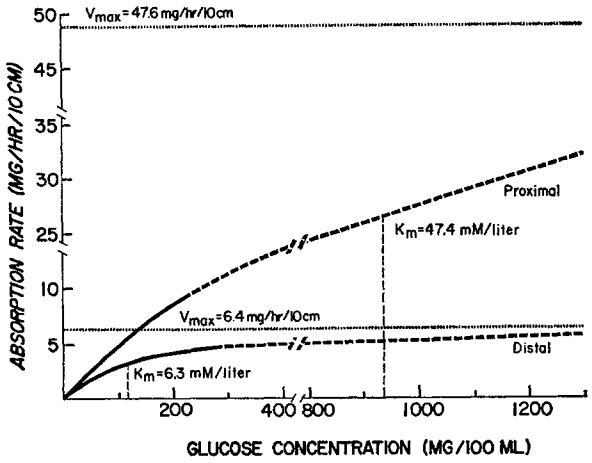

FIGURE 5. Comparison of the characteristics of the proximal and distal transport systems. Calculated K_M and V_{max} values are indicated by the dashed and dotted lines. K_M in the distal segment is within the range of the experimental data; i.e., solid portion of the curve. K_M in the proximal segment is on the extrapolated dashed portion of the curve, beyond the experimental data.

It may be assumed that the following processes are involved in glucose transport: (*a*) active transport; (*b*) carrier-mediated facilitated diffusion; (*c*) simple diffusion. Then, as a consequence of the nature of these processes, depending on relations of blood to lumen glucose concentrations, the proportions of transported glucose using the three pathways probably varied. At luminal glucose concentrations below those of blood, the absorption mechanism is active transport. Under these conditions facilitated and simple diffusion would make it possible for blood glucose to enter the lumen. Glucose could leave the lumen by simple and facilitated diffusion when luminal glucose concentrations were greater than those of blood. Thus, simple and facilitated diffusion could both decrease and increase glucose absorption in this study, depending upon the relationship of blood glucose to lumen glucose. This would decrease absorption at the first three concentrations and

increase it at the last two (Figs. 1 and 2). Thus, the form of the proximal absorption curve could reflect the net result of the interaction of these three glucose transport processes. It can be shown that this hypothesis does not completely explain the findings, since the experimental data can be used to obtain an estimate of the magnitude of simple and facilitated diffusion.

In these studies glucose-^{14}C was perfused with carrier glucose through the intestinal lumen. Blood glucose consisted chiefly of endogenous glucose, which was nonradioactive. Entry of blood glucose into the lumen would cause an increase in intraluminal glucose measured by the Somogyi method, but not by counting ^{14}C. This would lower absorption measured by the Somogyi method. In fact, proximal segment glucose absorption measured by the Somogyi method was 6–16% lower than by counting ^{14}C at the three lowest glucose concentrations. This is actually a good correlation between the two methods, but it is also an estimate of the rate of exchange between blood glucose and lumen glucose. As a measure of facilitated and simple diffusion of glucose, the rate is too low to explain the increased absorption in the proximal segment at the highest glucose concentration.

The failure of the rate curves to flatten was at first considered an artifact of the loading conditions. This possibility was tested by varying the rate of perfusion of the intestine. Glucose absorption rate was independent of a nearly threefold increase in rate of presentation of glucose to the proximal (Fig. 3 and Table I) and distal (Table II) small intestine. The concentration of glucose remaining within the gut lumen at the end of perfusion appears to provide sufficient glucose for transport (see Tables I and II, geometric mean glucose concentrations during perfusion at the slow and fast pump rates). The per cent absorption in the proximal small gut ranged from 57 (C_l25 mg/100 ml) to 40 (C_l600 mg/100 ml) at the slow pump speed to 28 (C_l25 mg/100 ml) to 23 (C_l300 mg/100 ml) at the fast pump speed. Similarly, the per cent absorption range in the distal small intestine was 43 to 16 and 30 to 10.

However, this does not exclude failure to achieve equilibrium at some step in the sequence of processes involved in glucose transport. Thus, at the perfusion rates used, flow through the intestine was laminar and luminal mixing may have been inadequate. Diffusion of glucose molecules from the axis of flow to the mucosa might not have been adequate to maintain the glucose concentration at the brush border. Intestinal blood flow is of the order of 3 ml/min/segment, assuming equal distribution of flow between the segments (8). Since there are several barriers to glucose movement between the brush border and the portal blood, an uneven distribution of glucose concentration in the mucosa as well as a failure to achieve intraluminal diffusion equilibrium is quite possible.This would have prevented saturation of rate-limiting steps in transport. It is unlikely that increasing intestinal perfusion rate to that of blood flow would avoid this. In vivo preparations for measuring absorption

under equilibrium conditions as well as instantaneous initial rates of individual steps are required. In unanesthetized preparations (1, 11) intestinal motility probably maintains adequate intraluminal mixing, and this may explain some of the differences from the results of this study. In the rat intestine in vitro (5) the lower absorption rates may have permitted saturation of rate-limiting steps even in the absence of motility.

These studies illustrate the difficulties in obtaining a controlled system for studying the physiology and biochemistry of transport in vivo. Although we found approximate Michaelis-Menten kinetics at low concentrations, over the whole concentration range this analysis is not applicable. The system is too complex for assignment of K_M and V_{max} values. The unique feature of the present investigation, measurement of absorption at intraluminal concentrations below and above those of blood glucose, cannot account for differences from previous studies.

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