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Effect of 5-Thio-D-glucose on Hexose Transport and Metabolism in the Mouse Small Intestine[1] (41852)

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Abstract. 5-Thio-D-glucose (5TG) elicits a time-dependent effect on net D-glucose transport and metabolism in the mouse small intestine. When incubation periods were less than 45 min, 5TG inhibited net D-glucose transport. As incubation periods were lengthened to 60 min or greater, net D-glucose transport was potentiated by 5TG, with a concomitant inhibition of lactate production from exogenous D-glucose. Furthermore, D-glucose inhibited 5TG transport at every time point studied. Initial velocity profiles done with 5TG and D-glucose, respectively, resulted in a K_T of 6.3 mM and a T_{max} of 18.5 μmole/g/30 min for 5TG; while for D-glucose the K_T was 11.7 mM and the T_{max} was 43.4 μmole/g/30 min. Competitive inhibition between 5TG and D-glucose was demonstrated. The apparent K_i's for 5TG and D-glucose were 3.0 mM and 9.3 mM, respectively. These findings indicated that 5TG has a lower affinity for the hexose-transport carrier than D-glucose. 5TG was also found to inhibit 3-*O*-methyl-D-glucose (3MG) and D-galactose transport in a dose-dependent manner. Basal levels of lactate production were not affected by 5TG nor did 5TG affect lactate levels in the presence of 3MG. These results suggest that 5TG decreases intestinal utilization of D-glucose via glycolysis to partially account for the observed increase in net transmural transport of D-glucose.

5-Thio-D-glucose (5TG), first synthesized by Feather and Whistler (1), is an analogue of D-glucose with sulfur substituted for oxygen in the pyranose ring. Rao *et al.* (2) found no evidence of important alteration in ring conformation of 5TG as compared to D-glucose. Using everted rings of hamster small intestine, Critchley *et al.* (3) and Barnett *et al.* (4) demonstrated that 5TG is accumulated against a concentration gradient. Little information is available concerning the transmural transport of 5TG and its effect on the transport and metabolism of D-glucose in the small intestine.

5TG was shown to inhibit several enzymes involved in D-glucose metabolism in rabbit skeletal muscle (5, 6) and to induce glycosuria and hyperglycemia when administered either orally or intraperitoneally to rats (7). Furthermore, we previously demonstrated that 5TG potentiates net D-glucose transport in the mouse small intestine (8). Since the small intestine is known to metabolize 50% of the D-glucose transported (9), 5TG may play an important role in the intestinal utilization of D-glucose and thus contribute to the diabetogenic effect of 5TG observed *in vivo* (7). Therefore the purpose of this investigation was to further characterize the transport process of 5TG and its effect on the transport and metabolism of hexoses in the small intestine *in vitro*.

Materials and Methods. *Materials.* 5-Thio-D-glucose, PGO enzymes, *o*-dianisidine diHCl, lactic acid, lactic acid dehydrogenase (LDH), NAD^+, Tris buffer, and meta-phosphoric acid were purchased from Sigma Chemical Company (St. Louis, Mo.). Radioisotopes were purchased from New England Nuclear Corporation (Boston, Mass.).

Animal treatment. Male Swiss–Webster mice purchased from Laboratory Supply Company (Indianapolis, Ind.) weighing 30 to 40 g were used in this study. The mice were maintained on stock diets and water *ad libitum* and a 12-hr light–dark cycle in quarters not sprayed with insecticides. Animals were sacrificed by cervical dislocation and decapitation. The abdomen was opened, then the jejunal section of the small intestine was removed and washed with Ringer's solution.

Intestinal transport of sugars. The everted intestinal sac technique (10) was employed to study the transmural transport of 5TG, D-glucose, 3-*O*-methyl-D-glucose (3MG), and D-ga-

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lactose. D-[^{14}C(U)]Glucose, 3-*O*-methyl-[D-^{14}C(U)]glucose, and D-[^{14}C(U)]galactose were the tracers used for analysis where specified. Both mucosal and serosal fluids were composed of mammalian Ringer solution, containing the same desired sugar(s) at identical specified concentrations. The sacs were incubated at 37°C in Dubnoff metabolic shaker, under 1 atm pressure of 95% O_2–5% CO_2 for desired intervals of time. At the end of the incubation, the sacs were removed, gently blotted on filter paper, and weighed. The serosal fluid was drained and centrifuged.

Analyses. When tracers were employed, a 50-μl serosal sample was pipetted into 10 ml of an aqueous scintillation fluid (11), prepared by combining 4 g of Omniflours (98% PPO and 2% Bis-MSB, New England Nuclear Corp.), 1 liter toluene, and 500 ml Triton X-100 (Rohm and Haas, Philadelphia, Pa.). Radioactivity was determined in an automatic liquid scintillation counter and concentrations were calculated from the specific activities of standard solutions.

D-Glucose was determined by a glucose oxidase method (Glucostat, Sigma Chemical Co.). 5TG was not found to be a substrate for this assay, in agreement with Hoffman and Whistler's finding (7). 5TG was estimated using the reducing sugar assay described by Nelson (12). L-Lactate was measured via an LDH assay (13).

Statistical analyses were performed by one-way analysis of variance with a Duncan's multiple-range test for significance. Regression lines were calculated by the least-squares method. The values are expressed as mean ± SE.

Results. *Transport studies.* The net transmural transport of D-glucose and 5TG across the mouse small intestine was linear as a function of time (Figs. 1A and B). Addition of D-glucose to 5TG caused an inhibition of 5TG transport. However, the transport of D-glucose in the presence of 5TG exhibited a biphasic effect. At time intervals less than 45 min, 5TG inhibited net D-glucose transport. If incubated for longer than 45 min, 5TG caused an enhanced rate of D-glucose transport.

Velocity profiles were done with 5TG and D-glucose, as substrates (Figs. 2A and B), respectively. The K_T for 5TG was found to be 6.3 mM and the T_{max} was 11.5 μmole/g/30 min; while D-glucose had a K_T of 11.7 mM and a T_{max} of 43.5 μmole/g/30 min. As indicated in Fig. 2, 5TG and D-glucose are competitive inhibitors of each other in terms of transport. Since the apparent T_{max} values for 5TG and D-glucose were so vastly different, we could not validly compare K_T values to estimate the relative affinity of either compound for the hexose-transport carrier. We, therefore, determined the apparent K_I values

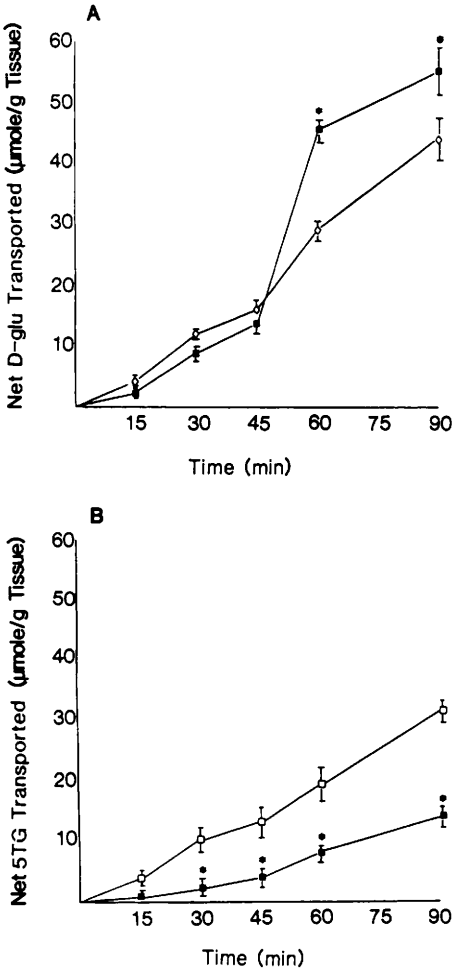

FIG. 1. Time profiles of D-glucose transport in the mouse small intestine. Values are the means ± SE of four experiments. (A) Net D-glucose transported at 5.5 mM alone (◇) and in the presence of 5.5 mM 5TG (■). *Denotes significantly different from D-glucose alone ($P < 0.01$). (B) Net 5TG transported at 5.5 mM alone (□) and in the presence of 5.5 mM D-glucose (■). *Denotes significantly different from 5TG alone ($P < 0.01$).

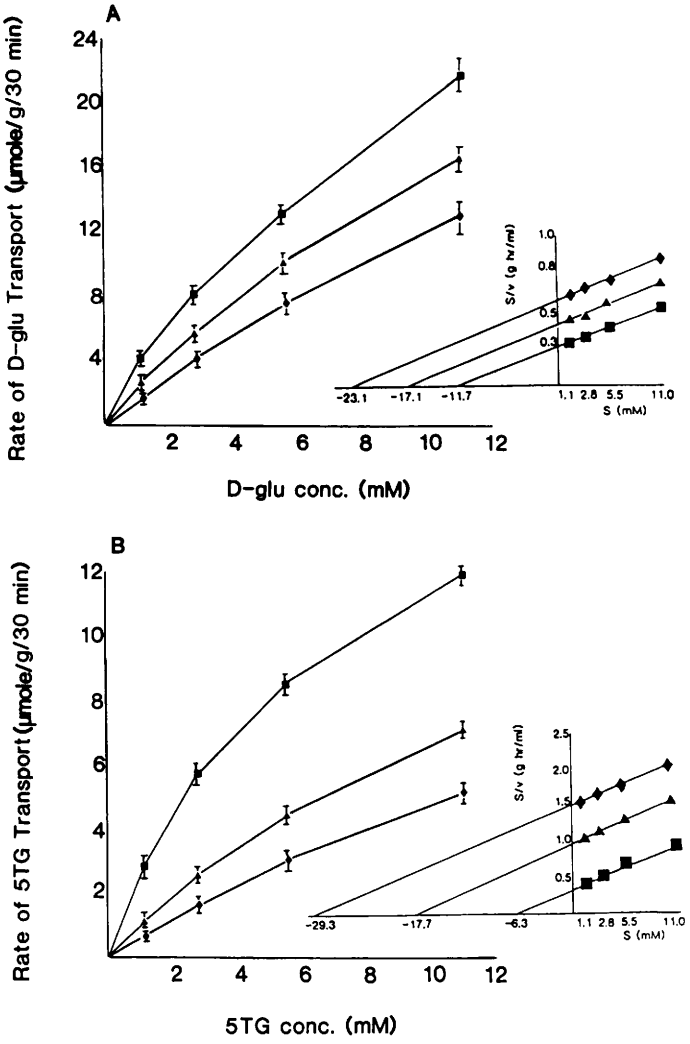

FIG. 2. Initial velocity profiles of D-glucose and 5TG in the mouse small intestine. Values are the means ± SE of four experiments. (A) Rate of D-glucose transport (■) and in the presence of 5.5 m*M* 5TG (▲) or 11.0 m*M* 5TG (♦). Insert: Hanes–Woolf plot of the above data. (B) Rate of 5TG transport (■) and in the presence of 5.5 m*M* D-glucose (▲) or 11.0 m*M* D-glucose (♦). Insert: Hanes–Woolf plot of the above data.

for both 5TG and D-glucose. Table I shows that 5TG has a K_I of 9.3 m*M*; while D-glucose has a K_I of 3.0 m*M*. This data demonstrated that D-glucose has a greater affinity for the hexose-transport carrier than 5TG.

Since 5TG enhanced the net transport of D-glucose after 45 min of incubation, the effect of 5TG on the transport of two other actively transported hexoses was investigated. As illustrated in Table II, 5TG inhibits the net transmural transport of both 3MG and D-galactose in a dose-dependent manner. The rates of 3MG transport were only 51 and 30% of control values in the presence of 5.5 and 11.0 m*M* 5TG. Likewise, the rates of D-galactose transport were 85 and 57% of control, respectively, in the presence of 5.5 and 11.0 m*M* 5TG. However, the apparent rates of D-glucose transport increased 85 and 114% over control values in the presence of 5.5 and 11.0 m*M* 5TG, respectively. This increase was dose-related although the effect of 5.5 and 11.0 m*M* 5TG was not significantly different from each other.

TABLE I. KINETIC-TRANSPORT PARAMETERS

Substrate	K_T (mM)	V_T (μmole/g/ 30 min)	K_I (mM)
5.5 mM D-glucose	11.7	43.5	—
5.5 mM D-glucose + 5.5 mM 5TG	17.1	41.7	9.3
5.5 mM D-glucose + 11.0 mM 5TG	23.1	40.0	9.3
5.5 mM 5TG	6.3	18.5	—
5.5 mM 5TG + 5.5 mM D-glucose	17.7	18.5	3.0
5.5 mM 5TG + 11.0 mM D-glucose	29.3	18.9	3.0

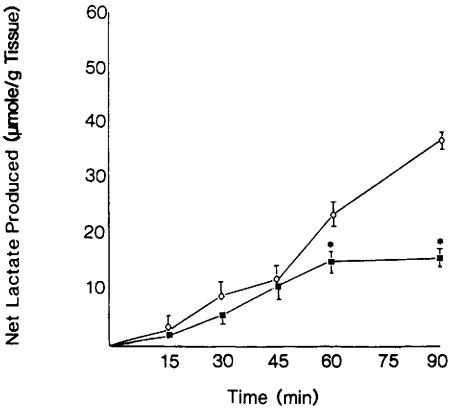

FIG. 3. Time profile of lactate production from exogenous D-glucose in the mouse small intestine. Values are the means ± SE of four experiments. In the presence of 5.5 mM D-glucose (◇) and 5.5 mM of both D-glucose and 5TG (■). *Indicates statistical significance of values compared to time-paired D-glucose alone ($P < 0.01$).

Metabolism. In the mouse small intestine, net lactate production from exogenous D-glucose was linear over time (Fig. 3). The addition of 5TG to D-glucose caused a significant inhibition of lactate production after 45 min of incubation (Fig. 3). As illustrated in Fig. 4, the rate of lactate production in the presence of 5TG (2.28 ± 0.61 μmole/g/hr) did not differ significantly from basal levels (6.55 ± 0.31 μmole/g/hr). Likewise, 5TG did not affect lactate production in the presence of 3MG (3.42 ± 0.42 μmole/g/hr), a nonmetabolizable sugar analogue. However, the rates of lactate production from exogenous D-glucose were inhibited 44 and 66% in the presence of 5.5 and 11.0 mM 5TG, respectively. Furthermore, we have found that 5TG is not converted to D-glucose, since D-glucose levels in the presence of 5TG did not differ from basal values (unpublished results).

Discussion. 5TG is an analogue of D-glucose which shares many chemical and physical properties (1). We have previously demonstrated that 5TG is actively transported across the mouse small intestine by a Na^+- and energy-dependent, phloridzin- and ouabain-sensitive mechanism (8). Critchley *et al.* (3) have shown competitive inhibition between 6-deoxy-D-glucose and 5TG, but to our knowledge no one has shown a direct interaction between D-glucose and 5TG transport. We demonstrated that D-glucose and 5TG are competitive inhibitors of each other, in terms of transport. Furthermore, the affinity of 5TG

TABLE II. EFFECT OF 5TG ON THE TRANSPORT OF VARIOUS ^{14}C-HEXOSES ACROSS THE MOUSE SMALL INTESTINE

	3MG		D-Galactose		D-Glucose	
Treatment	Rate of transport (μmole/g/hr)	% of control	Rate of transport (μmole/g/hr)	% of control	Rate of transport (μmole/g/hr)	% of control
Control[a]	22.86 ± 4.04[b] (14)	100	27.02 ± 1.98 (9)	100	29.62 ± 5.52 (11)	100
+5.5 mM 5TG	11.66 ± 2.28[c] (14)	51	22.96 ± 2.70[c] (10)	85	54.67 ± 4.52[c] (19)	185
+11.0 mM 5TG	6.84 ± 1.74[c,d] (8)	30	15.36 ± 2.22[c,d] (10)	57	63.48 ± 7.12[c] (19)	214

[a] 5.5 mM of test sugar alone.
[b] Mean ± SE, number in parenthesis is the sample size.
[c] Significantly different from control ($P < 0.01$).
[d] Significantly different from 5.5 mM 5TG ($P < 0.01$).

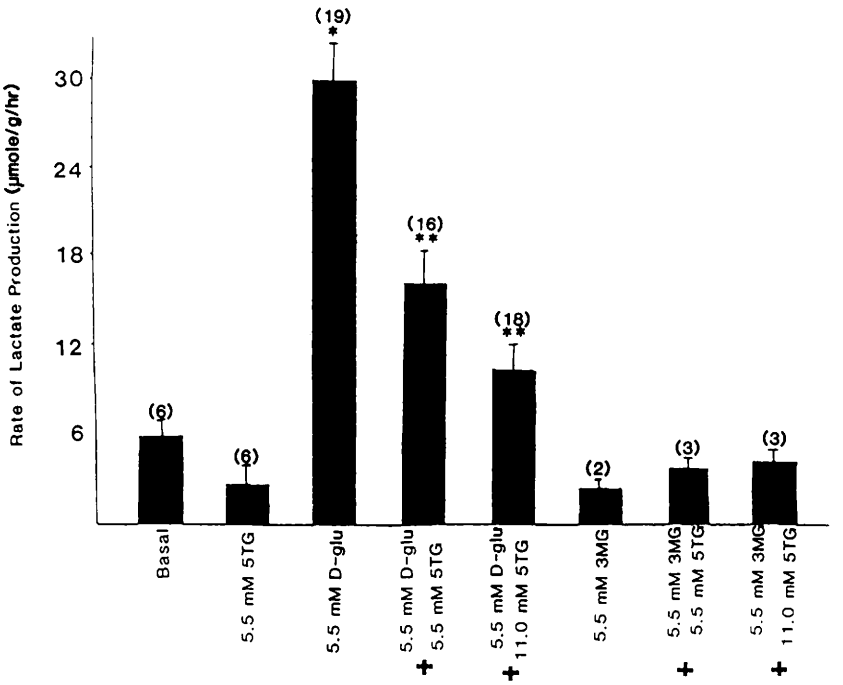

FIG. 4. Average rate of lactate production in the mouse small intestine under various conditions. Values are the means ± SE and the number in parenthesis is the sample size. *Denotes significantly different from basal levels ($P < 0.01$). **Denotes significantly different from basal levels and from 5.5 m*M* D-glucose ($P < 0.01$).

for the hexose-transport carrier was found to be lower than that of D-glucose in the mouse small intestine.

5TG elicited a biphasic effect on net D-glucose transport and a time-dependent inhibition of lactate production from exogenous D-glucose. The initial inhibition of D-glucose transport by 5TG is most likely due to competition at the hexose-transport carrier. The latter potentiation of net D-glucose transport by 5TG is presumably related to inhibition of D-glucose utilization in the intestine, since 5TG is not converted to D-glucose.

In addition, we demonstrated that 5TG inhibits 3MG and D-galactose transport, yet fails to affect lactate production in the presence of 3MG. The difference between potentiation and inhibition of net hexose transport by 5TG appears to relate to differences in hexose metabolism. Pritchard and Porteous (9) demonstrated that 50% of the D-glucose transported across the small intestine is metabolized, mainly to lactate (>90%). In contrast, it is well known that neither 3MG nor D-galactose is appreciably metabolized in the small intestine. Further evidence for a direct effect of 5TG on D-glucose metabolism by 5TG is obtained by Hellman *et al.* (14); where they demonstrated an 80% decrease in D-glucose metabolism by 5TG as measured by CO_2 production from pancreatic islets. Thus 5TG inhibited the intestinal utilization of D-glucose via glycolysis to partially account for the increase in net transmural transport of D-glucose.

It has been shown that 5TG is a potent inhibitor of phosphoglucomutase (K_I = 16.2 μM) as well as phosphorylase a and b, thereby blocking glycogen synthesis and degradation (5, 6). Pritchard and Porteous (9) demonstrated that intestinal glycogen stores were increased up to 130% of basal values in the presence of exogenous D-glucose. In addition, 10% or more of the glucose absorbed by the mucosal cells is oxidized via the hexosemonophosphate shunt (HMS) (15). 5TG-6-phosphate has been shown to be an inhibitor of glucose-6-phosphate dehydrogenase (5), which

is the rate-limiting step of the HMS (16). Our data (Figs. 3 and 4) demonstrated that 5TG inhibits D-glucose metabolism via glycolysis. However, it is possible that the inhibitory effects of 5TG on glycogen turnover and the HMS may also contribute to the increase in net transmural transport of D-glucose.

Preliminary studies by Hoffman and Whistler (7) demonstrated that 5TG is capable of eliciting a diabetogenic effect when administered either orally or intraperitonelly to rats. They suggest that the diabetogenic effect may be partially due to inhibition of D-glucose uptake in various tissues. We demonstrated that 5TG interacts with D-glucose at both the transport carrier and the metabolic pathways in the mouse small intestine. The interaction between 5TG and D-glucose demonstrated *in vitro* may also be a contributing factor for the diabetogenic effect of 5TG observed *in vivo*.

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