Renal and Intestinal Hexose Transport in Familial Glucose-Galactose Malabsorption

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ABSTRACT Glucose transport by jejunal mucosa in vitro and kidney in vivo was investigated in a 3 yr old patient with congenital glucose-galactose malabsorption, her family, and 16 normal volunteers. Glucose transport by normal human jejunal mucosa was concentrative, saturable, sodium and energy dependent, and exhibited competitive inhibition. Biopsy specimens from six normal controls and an asymptomatic 5 yr old brother of the proband accumulated glucose to concentrations 16 times that in the incubation medium. The proband's mucosa was unable to concentrate glucose throughout a 60 min incubation period. Both of her parents and a half sister demonstrated impaired glucose transport. Their values fell between normal and those of the proband. Influx of glucose was impaired but efflux of glucose from the mucosa of these three heterozygotes was identical with that in three normal controls. A kinetic analysis indicated a reduced capacity (V_{max}), but a normal affinity (K_m) for glucose transport by their intestinal mucosa. All subjects accumulated fructose similarly.

Renal glucose transport was investigated using renal glucose titration techniques. A partial defect in renal glucose reabsorption was found in the proband. Her brother's titration curve was similar to that of seven normal volunteers.

We conclude that familial glucose-galactose malabsorption is inherited as an autosomal recessive trait, that heterozygotes for this disorder are detectable and demonstrate a reduced capacity for glucose transport, and that absent intestinal glucose transport is accompanied by partial impairment of renal glucose transport.

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INTRODUCTION

14 patients have been reported previously with intestinal malabsorption of the sterically similar monosaccharides D-glucose and D-galactose (1–14). The familial nature of this disease was first determined by Lindquist and Meeuwisse in a study of one large Swedish family (2, 14, 15). They found first or second cousin relationships in three out of four patients, several instances of consanguineous matings, and six deaths caused by idiopathic diarrhea in siblings of affected patients. Subsequent authors have noted similar findings. No direct parent to child transmission has been reported and both sexes have been affected. Several cases demonstrated glycosuria (12). These previous observations suggested that the glucose-galactose malabsorption syndrome was inherited as an autosomal recessive trait in which both the kidney and intestine expressed an abnormality in glucose transport. However, no systematic studies of the mutant glucose transport processes have been undertaken in families affected with this disorder to test this genetic hypothesis.

In the present study, jejunal biopsy specimens were obtained and studied in vitro. To define the mode of inheritance of glucose-galactose malabsorption, several parameters of glucose tarnsport were studied in a 3 yr old girl, her parents, brother, and half sister. Glucose transport by normal human intestinal mucosa was characterized and compared to that in the patient and her family.

Like the jejunal epithelial cell, proximal renal tubular epithelium is differentiated histologically and functionally for the transport process. Three previously reported inherited diseases of amino acid transport have associated defects in the kidney and intestine (16–18). Therefore, in vivo renal glucose transport was also investigated by using renal glucose titration techniques to determine whether an intestinal defect was accompanied by impaired renal glucose transport.

METHODS

Patients studied. The proband, patient II-9 (Fig. 1) was the subject of a previous clinical report (13). Her clinical history included the onset of refractory diarrhea at 3 days of age which remitted only on a diet free of glucose and galactose. Carbohydrate was supplied as D-fructose, a monosaccharide transported by a different mechanism. Subsequent oral hexose tolerance tests confirmed that D-glucose and D-galactose were malabsorbed, but that D-xylose and D-fructose were absorbed normally. She was 3 yr old at the time of the present study. Although growth and development were proceeding normally on the glucose-free, fructose-supplemented diet, dietary glucose challenges continued to produce profuse watery diarrhea. The family history revealed that a sister, subject II-7, had died 2 yr earlier at 17 days of age of refractory diarrhea. The mother bore six normal children by a previous marriage, but her present marriage resulted in three children, two of whom were clinically affected. Intestinal and renal glucose transport was investigated in the proband (II-9), her asymptomatic 5 yr old brother (II-8), her parents (I-2, I-3), and her half sister (II-2). A total of 16 normal volunteers were also admitted to the Pediatric or Adult Clinical Research Center of the Yale-New Haven Hospital for intestinal or renal transport studies. Each patient was informed in detail of the investigative nature of the study and the procedures to be performed. In the proband (II-9) an oral glucose tolerance test (1.75 g/kg) resulted in watery diarrhea containing large amounts of glucose and an absent rise in blood glucose. Her intravenous glucose tolerance test and an oral D-xylose test were normal (19). Normal oral hexose tolerance tests were found in other family members.

Transport of glucose by human jejunal mucosa. A normal hematocrit, prothrombin time, partial thromboplastin time, and platelet count were obtained before accepting volunteers or patients for intestinal biopsy. In adults the Quinton 7 mm hydraulic intestinal multibiopsy apparatus[1] was used (20). The tube was swallowed and its tip placed at the ligament of Treitz under fluoroscopic control. 20 2–3 mg specimens of jejunal mucosa were obtained and placed in chilled Krebs-Ringer bicarbonate buffer for no more than 45 min before initiating transport studies. A Rubin tube was used in the proband and her brother, and four to eight jejunal specimens were obtained. Single biopsy specimens were placed in 2.0 ml of fresh buffer (pH 7.4) containing 2.0 mM D-glucose-U-^{14}C. The flasks were gassed with 95% O_2–5% CO_2 and incubated at 37°C for 5, 10, 30, or 60 min in a Dubnoff metabolic shaker. Specimens were incubated in separate flasks with D-fructose-U-^{14}C (2.0 mM) for 30 min. At the termination of the incubation, tissues were rinsed, weighed, and homogenized in 1.0 ml of a balanced barium hydroxide and zinc sulfate solution. Tissue homogenates were then centrifuged at 20,000 rpm for 10 min in a Sorvall RC-2B centrifuge.[2] Aliquots of medium were also precipitated with barium hydroxide and zinc sulfate. 0.2 ml of clear supernatant from tissues and media was prepared for liquid scintillation spectrometry. The distribution ratio of D-glucose-^{14}C or D-fructose-^{14}C (counts per minute per milliliter of extracellular fluid divided by counts per minute per milliliter of intracellular fluid) was calculated using previously described methods and tissue spaces (16). 0.5 ml of tissue and medium supernatant were analyzed for true glucose using a modified micromethod for glucose oxidase which

[1] Quinton Instruments, Seattle, Wash.

[2] Sorvall, Inc., Norwalk, Conn.

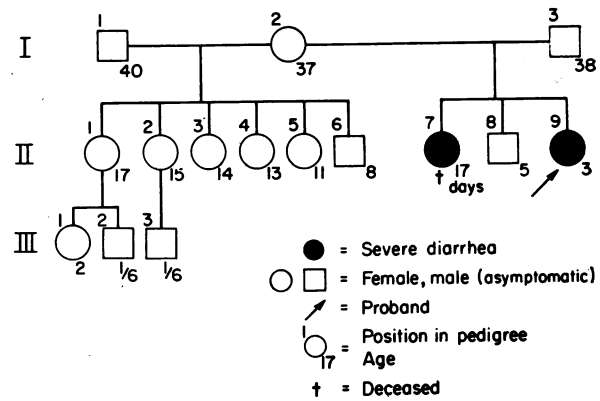


FIGURE 1 Pedigree of family affected with glucose-galactose malabsorption.

allowed accurate glucose determinations from 2.5 to 25 μg per sample. Tissue extracts, media, and standards were lyophilized and analyzed for purity by radiochromatography using a descending paper chromatographic system of isopropanol : water (160 : 40) or phenol : water (160 : 40). Sugars were identified colorimetrically by a diphenylamine : aniline reagent (21).

D-glucose-^{14}C uptake by biopsy specimens of jejunal mucosa was subjected to Michaelis-Menten analysis. At least 10 biopsy specimens per subject were incubated for 10 min in D-glucose-U-^{14}C ranging in concentration from 0.1 to 40.0 mM. The velocity of uptake was calculated from the distribution ratio, apparent diffusion constant (K_D), and medium concentration (A_f) using previously described techniques (22). The mean velocity of uptake from at least six observations at each substrate concentration was plotted for controls and compared to the mean velocities obtained in the parents (I-2, I-3) and half sister (II-2) of the proband. Using the double reciprocal plot of Lineweaver and Burk, the affinity (K_m) and capacity (V_{max}) in each group were observed directly from the extrapolated abscissa intercept and ordinate intercept (23).

D-glucose-^{14}C efflux from jejunal biopsy specimens was studied in the parents, half sister, and three normal volunteers. Two biopsy specimens were preincubated in one flask containing 2.0 ml Krebs-Ringer bicarbonate buffer and D-glucose-U-^{14}C (2.0 mM) for 30 min. At the end of this preincubation period, specimens were removed, rinsed, and placed in 2.0 ml of fresh buffer containing nonisotopic D-glucose (2 mM). At 5 min intervals from 0 to 30 min, 0.2 ml of medium was removed and placed in liquid scintillation vials. At the termination of the efflux study, tissues were analyzed by liquid scintillation spectrometry for remaining tissue radioactivity. The total number of D-glucose-^{14}C counts present in the tissue at the initation of the efflux study was estimated from the sum of the counts per minute per milliliter at each time interval of efflux and the number of counts remaining in the tissue at the end of the study. Efflux was expressed as the per cent of initial counts remaining in the tissue at each time interval.

The influence of 0°C, sodium-free buffer, ouabain (5×10^{-4} M), sodium cyanide (10^{-2} M), 2,4-dinitrophenol (10^{-4} M), D-galactose (40 mM and 10 mM), D-xylose (40 mM and 10 mM), and D-mannitol (40 mM and 10 mM) on D-glucose-^{14}C (2.0 mM) transport by jejunal biopsy specimens was studied in four normal, healthy volunteers. Sodium-free

buffer was prepared by substituting equal volumes of equimolar choline chloride for sodium chloride and Tris for sodium bicarbonate. Ouabain and the nonlabeled sugars were added at the initiation of a 30 min incubation period with 2.0 mM D-glucose-^{14}C. Tissues were incubated at 0°C or with sodium cyanide or dinitrophenol for 10 min before addition of D-glucose-^{14}C. The effect of these compounds or conditions was expressed as the mean per cent inhibition in each individual volunteer.

Nonradioactive D-glucose, D-fructose, D-xylose, and D-galactose were purchased from Fisher Scientific Company.[3] D-mannitol was obtained from Merck, Sharp, and Dohme.[4] D-glucose-U-^{14}C (specific activity 6.4 mCi/mmole) and D-fructose-U-^{14}C (4.0 mCi/mmole) were obtained from New New England Nuclear Corporation.[5]

Renal glucose titration studies. Seven normal volunteers, the proband (II-9), her brother (II-8), and her parents (I-2, I-3) underwent renal glucose titration studies using previously described techniques (24). After an overnight fast, each patient was given enough water to achieve a urinary flow rate of 10–20 ml/min. The children were sedated with meperidine hydrochloride (Demerol)[6] (1.5 mg/kg) and hydroxyzine (Vistaril)[7] (30 mg/kg) before introducing indwelling urinary and venous catheters. An inulin priming dose (50 mg/kg) was followed by a sustaining infusion of inulin (30 mg/min). After a 45 min equilibration period, duplicate 15-min clearance periods for inulin and glucose were obtained at fasting blood glucose levels. D-glucose was infused while continuing inulin infusion at the rate of 7.0 mg/kg per min, 15.0 mg/kg per min, and 30.0 mg/kg per min. Duplicate 15-min clearance periods were obtained after a 15 min equilibration period at each of these increasing filtered loads of D-glucose. Arterialized capillary blood samples (0.1 ml) were obtained from the finger tip by heating the hand in warm water (140°F) for 5 min. Urine samples were obtained from the adult patients without an indwelling bladder catheter. The preparation of protein-free filtrates of blood and urine, and the determination of D-glucose by a modified glucose oxidase method have been previously described (24). Glucose titration curves were constructed, and the minimal threshold ($Fmin_G$) and maximum tubular capacity for glucose reabsorption (Tm_G) were determined as previously described (24).

RESULTS

Characteristics of D-glucose transport by normal human jejunal mucosa. The results of D-glucose and D-glucose-U-^{14}C transport by biopsy specimens from six normal volunteers are shown in Fig. 2. Uptake increased with time. D-glucose-U-^{14}C was concentrated in the tissues to levels 14.4 and 17.5 times that in the medium at 30 and 60 min of incubation. More importantly, radioisotopic (clear bars) and enzymatic methods (stippled bars) yielded similar distribution ratios at all time points and radiochromatography of tissue extracts yielded a single homogeneous peak with an R_f identical with that of true glucose. These results indicate that the rate of intracellular catabolism of glucose by this tissue preparation is much slower than the rate of transport, and justify the use of radioisotopic methods in further kinetic analyses of the transport mechanism.

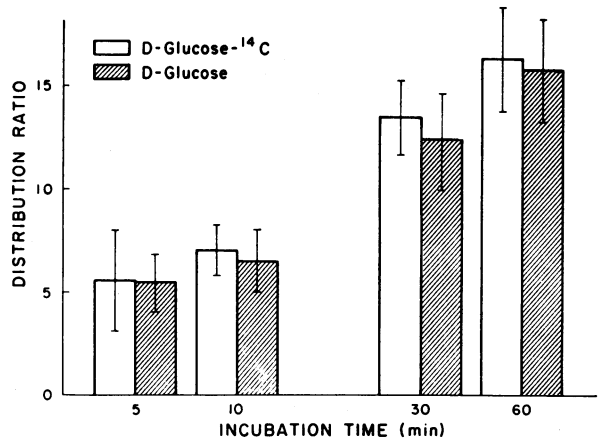


FIGURE 2 D-glucose and D-glucose-^{14}C (2.0 mM) transport by jejunal mucosa from normal volunteers (6). Individual biopsy specimens were incubated in Krebs-Ringer bicarbonate buffer, pH 7.4 at 37°C for times indicated on the abscissa. The mean distribution ratio for D-glucose-U-^{14}C (cpm/ml ICF)/(cpm/ml ECF) is indicated by clear bars and for unlabeled D-glucose (μg/ml ICF)/(μg/ml ECF) by stippled bars. The results are expressed as the mean of at least 12 observations bracketed by 1 SD at each incubation time.

The effect of certain metabolic inhibitors, and of the sodium ion of D-glucose transport by mucosa from normal volunteers is described in Table I. Marked inhibition of D-glucose accumulation was found when incubation

TABLE I

Effect of Metabolic Inhibitors and Sodium Ion Concentration on D-Glucose-U-^{14}C Transport by Jejunal Mucosa from Normal Volunteers

Experimental condition	Distribution ratio		Inhibition
	Control	Experimental	
	(cpm/ml ICF)/ (cpm/ml ECF)		%
Sodium-free medium	12.72	1.23	90.3
Ouabain (5×10^{-4} M)	12.72	1.96	84.6
0°C	13.25	0.51	96.2
Sodium cyanide (10^{-2} M)	31.72	0.55	97.5
2,4-dinitrophenol (10^{-4} M)	13.25	4.58	65.4

Single jejunal biopsy specimens were incubated for 30 min with D-glucose-U-^{14}C (2.0 mM). Results were expressed as the mean of at least triplicate observations. Each volunteer was used as his own control for the experimental condition indicated. Sodium-free medium was made by isotonic substitution of choline chloride for sodium chloride and Tris (hydroxymethyl aminomethane) for sodium bicarbonate. Tissues were incubated in the cold (0°C), sodium cyanide, or 2,4-dinitrophenol for 10 min before and during the succeeding uptake study. Values in parenthesis denote the concentration of inhibitor used.

[3] Fisher Scientific Company, Pittsburg, Pa.
[4] Merck, Sharp, and Dohme, West Point, Pa.
[5] New England Nuclear Corporation, Boston, Mass.
[6] Sterling Drug, Inc., New York.
[7] Pfizer Laboratories, New York.

TABLE II

Effect of Monosaccharides on D-*Glucose–U-*^{14}*C Transport by Jejunal Mucosa from Normal Volunteers*

Experimental condition	Distribution ratio		Inhibition
	Control	Experimental	
	(*cpm/ml ICF*)/(*cpm/ml EFC*)		%
D-galactose (40 mM)	15.71	2.11	86.6
D-galactose (10 mM)	21.73	6.81	68.7
D-xylose (40 mM)	13.25	14.77	—
D-xylose (10 mM)	13.25	15.41	—
D-mannitol (40 mM)	13.25	13.98	—
D-mannitol (10 mM)	13.25	14.25	—

The experimental conditions and expression of the data are identical with Table I. Figures in parenthesis indicate the medium concentration of the unlabeled monosaccharide added at the onset of a 30 min incubation period with D-glucose–U-^{14}C (2 mM). Results are expressed as the mean of at least duplicate observations.

was carried out in the absence of sodium ion in the incubation medium (90.3%) or in the presence of ouabain (84.6%). Active transport of D-glucose was also dependent on temperature and on energy derived from oxidative phosphorylation. When the tissues were exposed to cold (0°C), sodium cyanide, or 2,4-dinitrophenol before and during uptake studies, D-glucose transport was inhibited by 96.2, 97.5, and 65.4%, respectively. Competitive inhibition by sterically similar monosaccharides was also found (Table II). D-galactose inhibited D-glucose transport by 86.6% when present in 40 mM concentrations and by 68.7% when present at 10 mM. In contrast, the sterically dissimilar monosaccharides D-xylose and D-mannitol did not inhibit D-glucose uptake when present in either 10 or 40 mM concentrations.

Glucose transport by jejunal mucosa in glucose-galactose malabsorption. Jejunal biopsy specimens obtained from the child with glucose-galactose malabsorption and her family members were incubated in 2 mM D-glucose–^{14}C. The distribution ratios for each individual were calculated from radioisotopic (Fig. 3 A) and enzymatic (Fig. 3 B) assays. The proband was unable to accumulate D-glucose at any time throughout the 60 min incubation period. Her 5 yr old brother concentrated glucose to levels indistinguishable from normal controls. The distribution ratios attained by jejunal mucosa from both parents and the half sister fell between the values obtained in the patient and her normal brother. When uptake results in these three individuals were taken as a group and compared to those in normals at the 30 and 60 min time points, their distribution ratios were significantly reduced ($P < 0.01$ using the Wilcoxon-Rank-Sum test) (25). These results suggest autosomal recessive inheritance and indicate that the parents and half sister of the proband are heterozygous carriers for the mutant gene.

Efflux of D-*glucose from jejunal mucosa.* The following studies were performed to ascertain whether the diminished net uptake of D-glucose exhibited by the parents and half sister was caused by acceleration of efflux rather than impaired influx. When jejunal mucosa was preincubated with radioactive D-glucose–^{14}C (2.0 mM) for 30 min and transferred to isotope-free

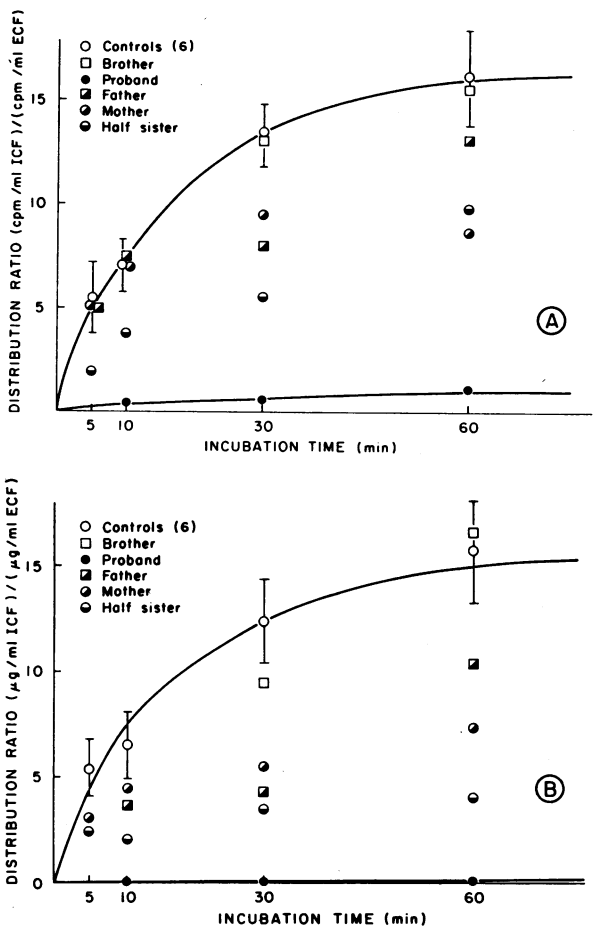


FIGURE 3 (A) D-glucose–^{14}C (2.0 mM) transport by jejunal mucosa in a family with glucose-galactose malabsorption. Incubation conditions and calculation of distribution ratios for D-glucose–^{14}C by liquid scintillation spectrometry are identical to those outlined in Fig. 2 and discussed in the text. Distribution ratios in the individual members of the pedigree represent the mean of at least two observations at each time of incubation. Normal values are those shown in Fig. 2. (B) D-glucose (2.0 mM) transport by jejunal mucosa. Incubation conditions and expression of the data are identical with part A. Distribution ratios for true D-glucose (µg/ml ICF)/(µg/ml ECF) were calculated from the same biopsy specimens used in part A.

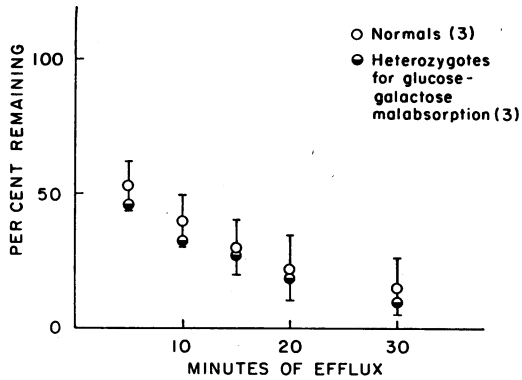


FIGURE 4 Efflux of D-glucose-^{14}C by jejunal mucosa. Tissues were preincubated for 30 min in buffer containing 2.0 mM D-glucose-^{14}C. Tissues were then removed, rinsed, blotted and placed in 2.0 ml of fresh buffer containing *unlabeled* D-glucose (2.0 mM). At the times indicated on the abscissa 0.2-ml aliquots of media were analyzed for radioactivity; the initial tissue radioactivity was calculated; and the per cent radioactivity remaining at each time interval was plotted on the ordinate. The results were expressed as the mean of six observations in three normal controls bracketed by 1 SD. The mean of six observations in the three heterozygotes was superimposed.

medium, a progressive increase in radioactivity in the medium was observed and the amount of radioactivity remaining in the tissue decreased with increasing time (Fig. 4). No difference in efflux was found in biopsy samples from the presumed heterozygotes when compared to normals, indicating that the observed impairment in glucose accumulation reflected slowed influx.

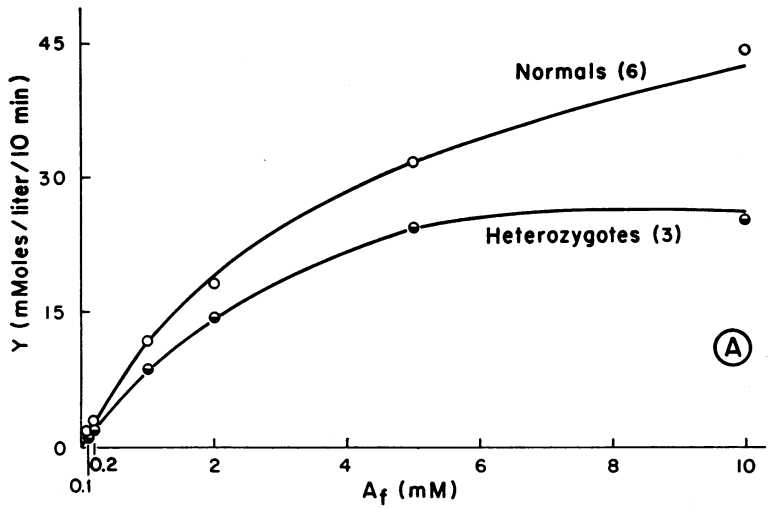
Initial rate of uptake of D-*glucose by jejunal mucosa.* To test the hypothesis of impaired influx and to clarify the mechanism of the transport defect, a kinetic analysis of initial D-glucose uptake by these heterozygotes was performed (Figs. 5 A and 5 B). Biopsy specimens were incubated for 10 min over a 400-fold range of glucose concentration. In Fig. 5 A the hyperbolic function of the velocity of mediated uptake (Y) versus the substrate concentration (A_f) was plotted. At all substrate concentrations above 2.0 mM, significantly impaired rates of transport were seen in the three heterozygotes ($P <$ 0.01 obtained by Wilcoxon-Rank-Sum test). When these data were analyzed by the double reciprocal method of Lineweaver and Burk (Fig. 5 B), a common extrapolated abscissal intercept, but different ordinate intercepts were seen. An apparent affinity constant (K_m) of 4.2 mM was obtained for both normals and the heterozygote. However, the normal capacity (V_{max}) of 55.5 mmoles/liter per 10 min was reduced to 41.7 mmoles/liter per 10 min in the heterozygotes.

Intestinal uptake of D-*fructose-^{14}C.* The steric specificity of the mutation was tested in vitro by investigating the uptake of D-fructose-^{14}C (2 mM). When this sterically dissimilar monosaccharide was incubated for 30 min with jejunal mucosa the final distribution ratio for carbon-14 was similar in controls, the proband (II-9), father (I-3), mother (I-2), and brother (II-8) (Fig. 6). Tissue homogenates were studied by descending paper chromatography; 27% of applied radioactivity was found within the glucose region, 50% in the fructose region, and 23% in an unidentified region running faster than D-fructose. The chromatographic distribution of tissue radioactivity was similar in four controls and the proband. Thus the normal distribution ratio of 3.5 did not indicate transport of D-fructose against a concentration gradient. The similar uptake and cellular distribution of radioactivity did suggest that transport and metabolism of fructose by jejunal mucosa was not impaired in glucose-galactose malabsorption.

Renal glucose titration studies. Because of the functional and histologic similarity between the jejunal mucosa and the proximal renal tubular epithelium, renal glucose transport was studied using in vivo glucose titration techniques. The results of these studies are summarized in Fig. 7 and Table III. The unaffected brother (II-8) demonstrated a normal titration curve. The affected proband (II-9) had an abnormal curve with a reduced minimal threshold ($Fmin_G$) of 82 mg/min per 1.73 m^2 (Fig. 8). She excreted more than 1 mg of glucose/min per 1.73 m^2 at all filtered loads of glucose above 82 mg/min per 1.73 m^2. This was clearly different from the mean $Fmin_G$ of seven normal controls and her brother's $Fmin_G$. However, her filtered load of glucose was inadequate to define her maximum reabsorptive capacity for glucose (Tm_G). At a maximum filtered load of 214 mg/min, she reabsorbed 187 mg/min, indicating that her Tm_G was only moderately reduced, if in fact it was depressed at all. The parents' titration curves were difficult to interpret. Although the mother's (I-2) $Fmin_G$ of 160 mg/min per 1.73 m^2 appeared reduced, it fell within 2 SD of seven normal controls. Her Tm_G of 341 mg-min per 1.73 m^2 was clearly normal. The father's (I-3) Tm_G was depressed below 2 SD of the normal mean and was obtained at a satisfactory filtered load of 442 mg/min per 1.73 m^2. His $Fmin_G$ was within normal limits. Neither the parents nor their daughter could be restudied to substantiate the abnormalities observed.

DISCUSSION

Previous studies using oral glucose tolerance tests failed to detect a defect in glucose absorption in parents of children with glucose-galactose malabsorption (13). Meeuwisse and Dahlqvist studied glucose transport by jejunal mucosa in vitro in the parents of an affected child (14). Their results suggest that the father, but



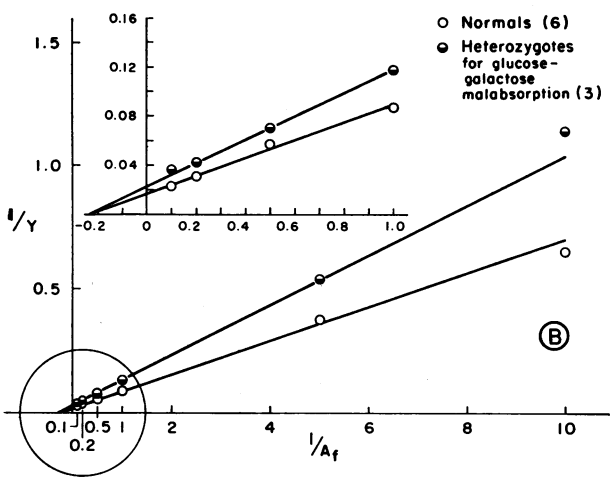


FIGURE 5 (A) Effect of increasing medium concentration (A_f) of D-glucose on the mediated uptake (Y) of D-glucose by jejunal mucosa from normal controls and heterozygotes for glucose-galactose malabsorption. Biopsy specimens were incubated for 10 min in D-glucose-U-^{14}C in concentrations from 0.1 to 40.0 mM. The velocity of mediated uptake (Y) was calculated from the apparent diffusion constant (K_D) and the observed distribution ratio. Each point represents the mean of at least four observations. Figures in parentheses indicate number of patients investigated. (B) Double reciprocal plot of data presented in part A. The circled portion indicates the values plotted on the expanded scale above.

not the mother, "might have a reduced rate of glucose absorption" (14). Our results demonstrate impaired intestinal glucose transport in both parents of an affected child and substantiate the following genetic hypothesis for glucose malabsorption in this family. The proband, who is unable to transport any glucose, is homozygous for a mutant gene producing defective hexose transport; her brother, who transports glucose normally, is homozygous for the normal allele at this locus; her parents and half sister, who accumulate glucose to intermediate levels are heterozygotes, each carrying one normal and one mutant gene. These findings are indicative of autosomal recessive inheritance.

Crane has demonstrated that glucose transport across the brush border of animal intestine involves at least two steps (26–29). The first step, binding of glucose to a component of the brush border is characterized by stereospecificity, competitive inhibition, and saturation kinetics. The second step concentrates glucose within

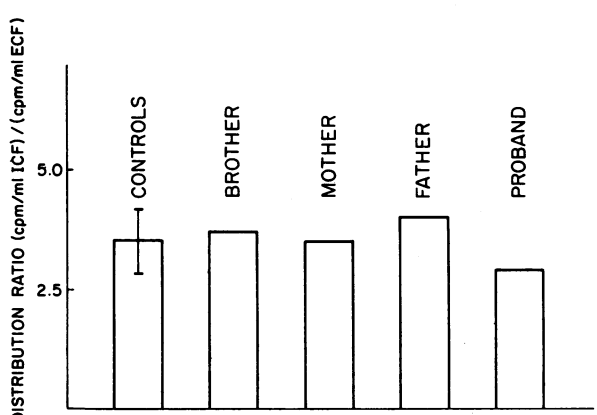


FIGURE 6 Uptake of D-fructose by jejunal mucosa. Tissues were incubated for 30 min in 2.0 mM D-fructose-^{14}C. Results in controls represent the mean of eight observations in four volunteers bracketed by 1 SD. Results from the family members are the average of two observations in each individual.

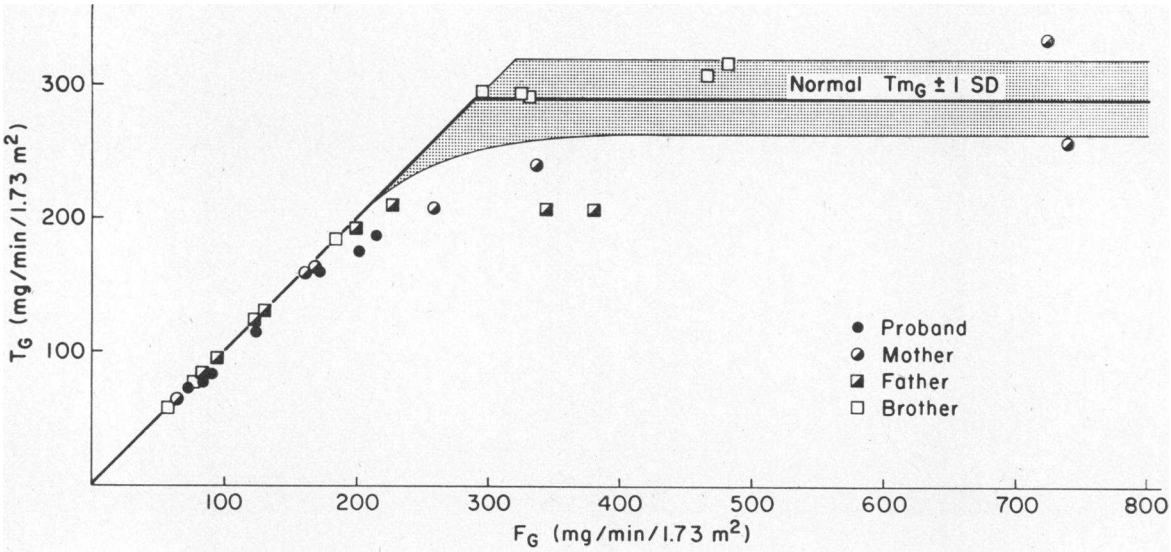


FIGURE 7 Renal glucose titration in a family with glucose-galactose malabsorption. Glucose reabsorption (T_G) is plotted against the filtered load of glucose (F_G). The solid black line denotes the mean titration curve in seven controls. The shaded area outlines 1 SD above and below the normal Tm_G.

the cell cytoplasm by mechanisms which are energy dependent and coupled to the outward transport of sodium ion. Our observations demonstrate many similarities between glucose transport in human and hamster intestinal mucosa. Glucose is transported against a concentration gradient into the jejunal mucosa. This concentrative step is inhibited by metabolic inhibitors such as cyanide dinitrophenol and cold. It is also inhibited by sterically similar (galactose) but not dissimilar (xylose and mannitol) monosaccharides. In addition, glucose transport by human jejunal mucosa is saturable and affinity and capacity for glucose transport are similar to hamster jejunum.

We have shown that the intestinal transport defect in

TABLE III

Renal Glucose Titration in the Glucose-Galactose Malabsorption Syndrome

Patients	Inulin clearance*	$Fmin_G$ ‡	Tm_G/C_{in}§	Tm_G‖	Fm_G/Tm_G¶
	ml/min per 1.73 m²	*mg/min per 1.73 m²*	*mg/ml*	*mg/min per 1.73 m²*	
Mother (I-2)	102	160	3.34	341	2.17
Father (I-3)	112	190	1.87	210	2.09
Brother (II-8)	140	300	2.29	319	1.51
Proband (II-9)	78	82	≧2.40**	≧187**	1.14
Normals	123 ±26	224 ±41	2.38 ±0.40	291 ±27	1.59 ±0.38

Figures in parentheses represent the position in the pedigree (Fig. 1). Normal values for $Fmin_G$, Tm_G/C_{in}, Tm_G, and Fm_G/Tm_G were expressed as the value of the mean ±1 SD from renal titration studies in seven normal adult volunteers (24).

* Inulin clearance (C_{in}) represented the mean of eight 15-min clearance periods.

‡ Minimal threshold for filtered glucose ($Fmin_G$) was the filtered glucose load in which significant amounts of glucose (> 1 mg/min) first appeared in the urine.

§ The mean threshold (Tm_G/C_{in}) was included to relate the tubular maximum for glucose reabsorption (Tm_G) to the glomerular filtration rate (C_{in}).

‖ The Tm_G is defined as the maximal observed rate of glucose reabsorption.

¶ Fm_G was the maximal filtered load of glucose.

** Because of the low Fm_G (214 mg/min per 1.73 m²) the true maximal rate of glucose reabsorption may be greater than the observed Tm_G.

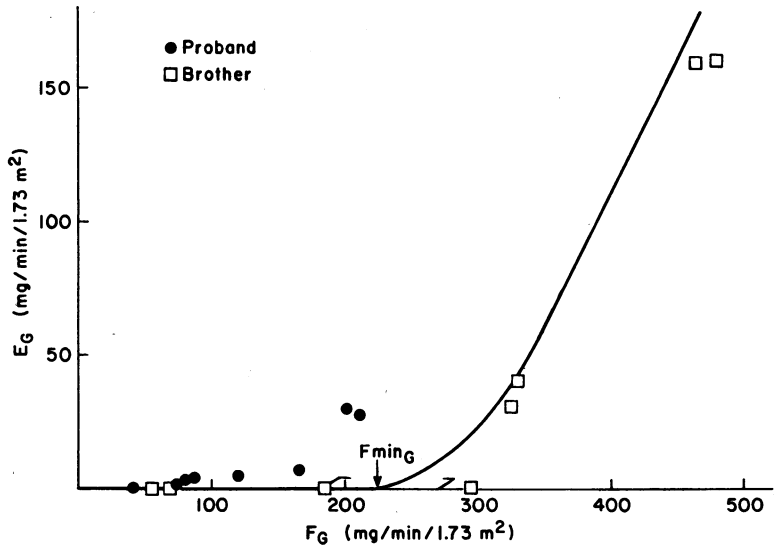


FIGURE 8 Relationship between filtered glucose load (F_G) and excreted glucose (E_G). The solid line represents the mean curve observed in seven normal controls. The mean minimal threshold ($Fmin_G$) is indicated by an arrow and bracketed by 1 SD.

glucose-galactose malabsorption is specific for the influx of D-glucose. Other workers have demonstrated that the disorder does not lead to a generalized aberration of energy-linked, sodium-dependent transport mechanisms. Eggermont, Meeuwisse, and their colleagues (9, 14) observed that intestinal biopsy specimens from patients affected with glucose-galactose malabsorption accumulated amino acids normally (9, 14). Since the accumulation of amino acids and hexoses into intestinal mucosa is sodium dependent, an abnormality in gradient-coupled sodium transport should affect uptake of amino acids as well as hexoses (30). More direct evidence suggests that sodium transport is normal. Meeuwisse demonstrated that ^{22}Na absorption in vivo and "pump ATPase" activity in vitro were normal in a patient with glucose-galactose malabsorption (14).

An abnormality at the glucose binding site is the most likely explanation for impaired glucose uptake. Several observations support this hypothesis. Recent direct evidence from microbial systems indicates that glucose binding to cell membranes involves a protein. Anraku identified and purified a glucose-galactose binding protein from the cell wall of *Escherichia coli* (K-12) which was under genetic control. This purified protein is stereospecific for glucose and galactose, and bacterial mutants for this protein exhibit a reduced rate of galactose uptake (31–33). It is reasonable to suggest that similar genetically controlled protein carrier(s) for monosaccharides might be present in man. Meeuwisse found that phlorizin, a competitive inhibitor of glucose tranpsort, failed to further decrease impaired glucose uptake by jejunal mucosa from an affected patient (14). Schneider, Kinter, and Stirling using radioautography, found absent binding of D-galactose-^{14}C and phlorizin-^{3}H to a brush border preparation from another patient (7). Despite these observations, no structural barrier or morphologic abnormality has been seen by either light or electron microscopy of the brush border from affected patients (14). Our kinetic analysis of glucose transport by heterozygotes indicates that a single mutant glucose transport gene results in a transport mechanism with reduced capacity (V_{max}), but normal affinity (K_m). This suggests a reduced number of functioning glucose binding sites, but no direct evidence for such a thesis is available in mammalian systems at this time.

The presence of abnormal renal as well as intestinal glucose transport offers further evidence for a genetically determined abnormality. Inborn errors of amino acid transport have previously demonstrated this correlation. In cystinuria, Hartnup disease, and iminoglycinuria a defect in renal tubular transport is often accompanied by impaired intestinal transport (16–18). Our study indicates that absent glucose binding by the proband's intestinal mucosa is accompanied by only a partial defect in renal glucose transport. This finding suggests that the kidney contains glucose transport systems in addition to those in the intestine. In another inherited disease of glucose transport, renal glycosuria, a severe defect in the kidney, is unaccompanied by a defect in the intestine (24). Similarly, a mutant gene which im-

pairs renal transport of glycine and the imino acids may not affect intestinal accumulation of these amino acids (34). Since patients with glucose-galactose malabsorption have normal intravenous glucose tolerance tests, it seems likely that the expression of this mutation is confined to gut and kidney, two tissues which have differentiated for transport. However, direct examination of transport in other tissues such as erythrocytes, leukocytes, and fibroblasts are needed to verify this assumption.

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