IN THE 1960s, two milestones were reached in the physiology of intestinal sugar absorption. The first was the Na$^+$-glucose cotransport hypothesis of Crane and colleagues (1), which explained active sugar transport, and the second was the discovery of glucose and galactose malabsorption (GGM) in patients (5, 6). The cotransport hypothesis has been exhaustively tested, confirmed, and extended to include the “active transport” of a wide variety of substrates into cells, ranging from lactose accumulation in Escherichia coli to iodide accumulation in the thyroid gland. Essentially, cotransporters are molecular machines that use energy stored in the form of ion electrochemical potential gradients across cell membranes, Na$^+$ or H$^+$, to drive the accumulation of specific solutes and water in cells (22). The intestinal Na$^+$-glucose cotransporter (SGLT1) uses Na$^+$ and electrical gradients across the membrane to drive sugar and water into enterocytes against their concentration gradients (9, 13, 23). Glucose and galactose are both handled by SGLT1, whereas fructose is transported across the brush border by its own private carrier, the facilitated fructose transporter (GLUT5). Glucose, galactose, and fructose complete their journey across the cell into blood through another facilitated sugar transporter (GLUT2) in the basolateral membrane (Fig. 1).

GGM is characterized by neonatal onset of watery and acidic severe diarrhea, which is fatal within a few weeks unless lactose (glucose and galactose) is removed from the diet (2). The diarrhea stops with fasting or withdrawal of the offending sugars from the diet but promptly resumes with oral feeding of diets containing lactose, glucose, or galactose. Fructose absorption is unaffected. Given the symptoms of the disease and what was known about intestinal sugar absorption at the time, it was predicted that GGM was due to a defect in the brush-border Na$^+$-glucose cotransporter. This hypothesis was strengthened by the exquisite autoradiographic galactose uptake and phlorizin binding experiments done on mucosal biopsies from the first American GGM patient (17, 18). These experiments showed that the reduction in galactose transport was associated with a 90% decrease in phlorizin binding to the brush border. Phlorizin is a specific, nontransported, competitive inhibitor of SGLT1.

The most reliable diagnostic test for GGM is the H$^2$ breath test (Fig. 2). Oral administration of glucose or galactose (2 g/kg) results in breath H$^2$ elevation greatly above 20 parts/million in patients with GGM but no such increase in controls or patients fed fructose. Children with GGM thrive “normally” on fructose replacement formulas, but symptoms return even in adulthood with as little as a teaspoon of glucose (6 g), and the H$^2$ breath test remains positive. The disease is quite rare. We are aware of about 200 patients worldwide, and a high proportion of cases are from consanguineous relationships.

The physiology and pathophysiology of intestinal sugar absorption were advanced in 1987 by our coworkers (Coady, Ikeda, and Turk et al. (20) of the first mutation in a transporter to cause a genetic disease, GGM. We obtained intestinal biopsies from two sisters diagnosed with GGM and blood samples from the parents who are cousins. Turk et al. (20) identified a homozygous, missense mutation (Asp28Asn) in the SGLT1 cDNA from each sister, found that each parent was a carrier for this mutation, and demonstrated that indeed the mutation completely abolished Na$^+$-glucose cotransporter using an oocyte expression assay. In the same kindred, prenatal screening was subsequently carried out on two fetuses and one (the
probands sibling) was found to be a carrier for the Asp28Asn mutation and the other (a cousin) was found to be normal. Both children have thrived without dietary restriction and remained asymptomatic for at least two years (11).

Further progress was initially hampered by difficulty obtaining mucosal biopsy samples from children with GGM until Turk et al. (21) succeeded in mapping the entire human SGLT1 gene. The gene is large, with 15 exons distributed among 72 kb of DNA. Once exons and their flanking regions were sequenced, a single-stranded conformational polymorphism assay to screen patients for mutations using genomic DNA from a small blood sample was developed. This development involved PCR amplifying each of the 15 exons and their intron-exon junctions and gel electrophoresis of the denatured PCR products to identify exons carrying mutations. Anomalous exons were then sequenced. To determine if the mutations were responsible for the defect in sugar transport, the mutants in Xenopus laevis oocytes were expressed and then biophysical and biochemical methods were used to determine the level of protein in the cell and in the plasma membrane. In cases in which the transporter was inserted into the plasma membrane, we (7) examined the partial reactions of the transport cycle. We were initially disappointed to find that with the first 21 missense mutants studied the primary defect was due to mistrafficking of the transporters in the cell. On the basis of Western blots, all the mutants were synthesized at levels similar to, or higher than, the wild-type SGLT. However, charge measurements (7) and freeze-fracture electron microscopy (24) of the oocyte plasma membrane demonstrated that there was a severe reduction in the number of cotransporters in the plasma membrane (10, 12). As judged by the degree of core and complex glycosylation of the mutants, the defect in trafficking of SGLT1 to the plasma membrane occurred between either the endoplasmic reticulum and the Golgi or the Golgi and the plasma membrane. Misfolding of mutant proteins may be the primary cause of transporter misorting (19). In only one case, Gln457Arg, was the mutant protein in the oocyte plasma membrane near normal levels.

What relevance do these experiments on oocytes have to the intestines of GGM patients? To answer this, we examined (unpublished data) the distribution of SGLT1 protein by immunocytochemistry in the mucosal biopsies from three patients with homozygous mutations. In all three, the distribution of the mutant proteins in the oocyte was identical to the distribution of SGLT1 in the patient's enterocytes: in two the protein was in the cytoplasm, and in one the protein was in the brush border. There is also agreement between our results on oocytes and those obtained by autoradiographic studies of biopsies from the first American GGM patient (18). Stirling and his collaborators (18) found that the binding of phlorizin to the patient's brush border was reduced by 90%, and we found no mutant SGLT1 protein (Cys355Ser and Leu147Arg) in the oocyte plasma membrane (10). These studies suggest that, at least with these four GGM mutants, the oocyte recapitulates the behavior of the mutant protein in the enterocyte.

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A major remaining question is how the missense mutations distributed throughout the protein (Fig. 3) disturb trafficking of the transporter to the plasma membrane. Answers to this question are important in understanding the biosynthesis of plasma membrane proteins and in devising improved therapies for children with GGM.

The GGM mutation in one kindred, Gln457Arg, has provided invaluable insight into the mechanism of sugar transport. Lostao studied (in preparation) the behavior of Q457R SGLT1 expressed in oocytes and the patient's intestinal mucosa and found that the protein is translated, glycosylated, and inserted into the plasma membrane, but it is unable to transport sugar. In the absence of sugar, the mutant protein transports Na⁺ by the Na⁺ leak or Na⁺ uniport pathway, and this is blocked by phlorizin. Glucose also is an inhibitor because it also blocks this Na⁺ transport pathway, indicating that glucose binds to Q457R SGLT1 but that it is not transported, i.e., the mutation produces a sugar translocation defect. Panayotova-Heiermann and colleagues (15) independently demonstrated that the sugar “pore” through SGLT1 is formed by the COOH-terminal domain of SGLT1 bearing residue Q457.

To exploit these observations we [Loo, Hirayama, Gallardo, and Lam (8)] have examined the role of Q457 in sugar translocation. In this study, the cysteine mutant Q457C was found to retain full Na⁺-glucose transport activity, apart from an increase in the apparent glucose Michaelis-Menten constant ($K_m$) from 0.4 to 6 mM, and chemical mutagenesis of Q457C with either charged or neutral alkylating reagents (methanethiosulfonates, MTS) was found to fully block sugar transport. However, because the alkylated Q457C protein binds glucose with a dissociation constant very similar to the apparent $K_m$ for sugar transport by Q457C SGLT1, this residue must not be part of the sugar binding site. The inhibition of sugar transport by Q457C by MTS only occurred when the cotransporter was in the outward-facing Na⁺ conformation, C2 (Fig. 4). The reagent was
not effective in the absence of Na+, in the presence of Na+ and glucose (or phlorizin), or in the presence of Na+ at depolarized membrane potentials. Voltage-jump experiments with rhodamine-labeled Q457C also showed that the time course and level of fluorescence closely followed the transition of the cotransporter between conformations C2 and C6 (Fig. 4). We interpret these results to mean that the cotransporter can exist in at least three different conformations (C6, C2, and C3) and that the coupling between Na+ and sugar transport occurs through ligand and voltage-induced conformational changes in the protein.

Preliminary studies with two other GGM missense mutations in the COOH-terminal domain of SGLT1, A468V and R499H (Fig. 3), show that replacing the residues with cysteines restores trafficking of the protein to the oocyte plasma membrane. Both proteins are functional, and sugar transport is blocked by MTSl reagents. As in the case of Q457C, these residues are accessible to MTSl reagents only when the proteins are in the C2 conformation. These results support my view that transmembrane helices 10–13 (Fig. 3) form the sugar pore. Further work is needed to identify the Na+-pore.

In summary, molecular biology studies on SGLT1 have led to the cloning of the cDNA for the human SGLT1 and the mapping of the gene, which have provided powerful new tools to examine the physiology of Na+-glucose cotransport and to study GGM. GGM has been confirmed to be due to mutations in the SGLT1 gene, and most of these mutations result in either truncated SGLT1 protein or mistrafficking of the transporter in the cell. As anticipated for an autosomal recessive disease, a private mutation produces the disease in each kindred and the frequency of the disease increases in cultures with a high frequency of consanguineous marriages. Although GGM is rare, it is possible that the larger pool of individuals carrying mild SGLT1 mutations, or severe mutations on one allele, has impaired glucose and galactose absorption. About 10% of the normal population, medical students, gave positive glucose H2 breath tests (14). This interface between physiology and disease has not only increased the understanding of the pathophysiology of sugar absorption but has provided new approaches to study the molecular mechanisms of the coupling between Na+ and sugar transport across plasma membranes.

These advances in SGLT1 and GGM studies would not have been possible without the superb contributions of talented members of this laboratory over the past 12 years, the physicians around the world who were generous in providing specimens from their GGM patients, and support from National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-19560, DK-44582, and DK-44602. The restrictions on Na+-glucose cotransport defects leading to renal glycosuria and congenital selective intestinal malabsorption of glucose and galactose. In: Metabolic Basis of Inherited Disease (7th ed.), edited by C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle. New York: McGraw-Hill, 1994, p. 3563–3580.

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