Problems of Transfer of Carbohydrates at the Level of the Intestinal Mucosa

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In humans, about half the daily energy is ingested as carbohydrates. Most have to be hydrolyzed by the salivary and pancreatic α-amylases and/or the brush border glycosidases of the enterocytes. The end products of the intestinal digestion of the dietary carbohydrates are glucose, galactose, and fructose (1,2). The resorption of the end products and not the hydrolysis of the oligosaccharides is the limiting step for all disaccharides except lactose and the α-1,6-oligosaccharides. Hydrolysis of oligomers larger than maltohexaose is rate limiting for glucose absorption in the absence of luminal α-amylase activity (3). Although most of the hexoses are absorbed in the small intestine, the colon has some salvage function (4).

In this chapter I shall review (1) some aspects of normal and abnormal absorption of glucose, galactose, and fructose; (2) the molecular basis of the absorption of glucose; and (3) the genetic control of the glucose carrier.

NORMAL AND ABNORMAL ABSORPTION OF HEXOSES IN THE SMALL INTESTINE

From 1960 on, Robert Crane and co-workers presented increasing evidence that the small intestine is able to absorb glucose and galactose, but not fructose, against a concentration gradient (5,6). At about the same time, Lindquist & Meeuwisse (7) and Laplane et al. (8) independently described infant patients with profuse watery diarrhea from birth following the ingestion of milk. Because the diarrhea ceased if carbohydrate-free feeds were offered or if fructose was substituted for glucose and galactose in the diet, the authors proposed that the affected infants suffered from a specific abnormality of glucose and galactose absorption. For these reasons the normal and abnormal absorption of glucose and galactose and of fructose is discussed separately.
TABLE 1. Study of active transport in small intestinal biopsy specimens in normal and glucose-galactose-intolerant children

<table>
<thead>
<tr>
<th></th>
<th>Relative tissue concentration (C/Cm)</th>
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<tbody>
<tr>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td>Na&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control subjects</td>
<td>6.35 ± 2.33</td>
</tr>
<tr>
<td>(Number of subjects)</td>
<td>(n = 12)</td>
</tr>
<tr>
<td>Glucose-galactose-intolerant patients</td>
<td>0.83 ± 0.42</td>
</tr>
<tr>
<td>(Number of subjects)</td>
<td>(n = 5)</td>
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</table>

*The active transport has been studied according to the technique described by E. Eggermont and H. Loeb (45). Active transport is found whenever the biopsy specimen is able to achieve higher concentrations of the substrate (C) than present in the incubation medium (Cm). Glucose is studied in the presence of Na<sup>+</sup> or in the absence of Na<sup>+</sup> replaced by K<sup>+</sup>. The accumulation of leucine, in the presence of Na<sup>+</sup>, is studied as a control.

Absorption and Malabsorption of Glucose and Galactose

In the adult, the absorption of monosaccharides from the jejunum has been studied by perfusion techniques (9). It has been shown that as the concentration of glucose or galactose in the perfusion fluid is increased, the absorption rate of glucose and galactose approaches a maximum. An apparent maximum velocity (V<sub>max</sub>) and half-saturation concentration (K<sub>m</sub>, Michaelis constant) can be calculated. These are for glucose V<sub>max</sub> 0.73 g/cm·min, K<sub>m</sub> 220 mM (4 g/dl), and for galactose V<sub>max</sub> 1 g/30 cm·min, K<sub>m</sub> 330 mM or 5.9 g/dl. In the young infant, we found an absorption of approximately 0.235 g glucose per 30 cm·min at a glucose concentration of 550 mM or 10 g/dl (10). The development of d-glucose absorption in the perinatal period has been studied by McNeish et al. (11).

In infants with glucose-galactose malabsorption, the reduced uptake of glucose (0.080 g/30 cm·min) together with the normal absorption of fructose (0.261 g/30 cm·min) could directly be demonstrated by disappearance of the monosaccharides from a 10% solution continuously infused into the lumen (10). Quantitative investigations on the residual capacity of glucose and galactose absorption in patients with glucose-galactose malabsorption were performed by several authors using the perfusion technique. Although glucose concentrations differed widely in the infusion solutions (between 11 and 556 mM), the absorption ratios were similarly low, 6–15% of the intake (12). The residual absorption of glucose and galactose in these patients is similar to the uptake of mannitol and may, therefore, be attributed to a passive process based on diffusion. With the use of radioactive sugars, Linneweh et al. demonstrated that the uptake of glucose or galactose did not exceed 10% of the administered dose, whereas the normal child resorbed over 90% (13).

Patients with glucose-galactose malabsorption also have a renal tubular transport
maximum for glucose (T_m G/1.73 m²) of no more than 25% of adult normal values, with an apparent inability to maintain this low level of function with prolonged increase in the filtered load (14). Although there are several indications that glucose can be absorbed from the lumen of the fetal lung by active mechanisms, glucose-galactose malabsorption seems to have no clinical expression during fetal life (15).

Infants and children suffering from glucose-galactose malabsorption can be treated effectively with a diet free of these monosaccharides (16,17). As only about 50 patients have been described in published reports, good follow-up studies are lacking. However, some clinical remission seems to occur with increased age even though active jejunal glucose transport remains absent. The intestinal absorptive surface area and intraluminal volume probably increase with increasing age, thereby reducing the effects of malabsorbed glucose on water secretion and intestinal motility (18).

Absorption and Malabsorption of Fructose

The absorption rate of fructose, on the other hand, is directly proportional to its concentration in the infused fluid. If the rate of glucose absorption is taken as 100%, then from 1 g/100 ml sugar solution the relative rate of fructose absorption is 64%, while from 5 g/100 ml solution it is as high as 89% (9). For these reasons, fructose absorption in humans seems to take place by energy-independent facilitated transport, although an active transport has been found in the rat small intestine (19). Recently, intestinal α-fructose absorption has been investigated using measurements of breath hydrogen, and was frequently found to be incomplete both in children (20) and in adults (21–23). Incomplete absorption may be associated with symptoms of cramps and diarrhea and may occur after the ingestion of as little as 5 g of fructose, even in the adult (22).

Glucose, however, which stimulates fructose uptake in a dose-dependent fashion, may abolish the intolerance, although apple juice, which contains fructose in excess of glucose, might induce abdominal symptoms in susceptible children (20). Barnes et al. reported on a patient aged 12 years, in whom challenge with as little as 1 g of fructose was followed by watery bowel movements and some abdominal discomfort (24).

Secondary Monosaccharide Intolerance

This condition occurs only in young infants and despite its temporary state, may be life threatening. The diarrhea stops only with the removal of all sugars, including fructose, from the diet. The jejunal morphology and the enzymic activities may either be normal or disturbed. Secondary monosaccharide intolerance is tentatively attributed to a "contaminated small bowel." Therefore, the condition is frequently associated with small intestinal obstruction or protracted gastroenteritis (25).
GLUCOSE-GALACTOSE CARRIER

As almost no information is available on the fructose carrier, the present discussion will be limited to the properties of the glucose-galactose carrier operating in the brush border membrane of the small intestine.

In 1958, Riklis and Quastel made the observation that the intestinal movement of glucose is dependent on the presence of Na⁺ in the medium (26). In 1962, Bihler et al. showed that reduction in tissue energy supplies eliminates the accumulation of glucose against a concentration gradient but not the Na⁺-dependent equilibration of glucose (27). They postulated that the sugar carrier possesses a binding site for cations and later demonstrated that in the presence of Na⁺, the carrier manifests about 100 times more affinity for its substrate than in the presence of K⁺ (28,29). Since the carrier is assumed to be exposed alternately to the luminal content, rich in Na⁺, and to the cytoplasm, low in Na⁺ and rich in K⁺, the sugar can be transported against its concentration gradient because of ionic differences. The perpetuation of the active transport of glucose will, however, depend on the continuous extrusion of Na⁺ by the (Na⁺/K⁺) -activated ATPase located in the serosa facing membranes (30).

In recent years, the D-glucose transport system has been studied extensively in vitro with the use of intact small intestinal tissue preparations or isolated brush border membrane vesicles. The state of the art can be found in two review articles written by Giorgio Semenza who has made major progress in this field (31,32). At present, there is evidence that, apart from the capacity for diffusion (33), the brush border membrane is endowed with two Na⁺, D-glucose cotransporters (31,34). The two systems are already operating in the jejunum and ileum of 17- to 20-week-old human fetuses (35). With the use of brush border membrane vesicles from guinea pig jejunum and with D-glucose as substrate, Alvarado et al. could demonstrate that at low glucose concentrations (1 mM or less) most of the uptake occurs through cotransporter-1; this system saturates rapidly and becomes practically constant at about 4 mM glucose (34). Although cotransporter-2 has a much lower affinity (25 mM versus 0.5 mM), its V_max is much larger (2300 pmol/mg protein·s versus 340). Hence, starting at about 4 mM glucose, the contribution of the cotransporter-2 to the total uptake increases steadily and a plateau is reached only around 100 mM glucose. Therefore, the two systems function as a tandem: cotransporter-2 takes care of the initial, rapid processing of the sugar load and cotransporter-1 takes over later so that no free sugar remains in the lumen during periods of fast. As concerns simple diffusion, this is negligible at low substrate concentrations but increases rapidly, so that at concentrations of 75 mM or higher (34) its contribution to total glucose uptake exceeds that of cotransporter-1.

The Na⁺, D-glucose cotransporter is inserted asymmetrically in the brush border membrane and has asymmetric functional properties. The transport agency does show kinetic asymmetric gated channel characteristics, and its function is probably related to protein structure fluctuation (35,36). The D-glucose transport is stimulated by Na⁺ only, and other monovalent cations have no effect (37). The positive charge
associated with Na⁺ is not compensated by the co-movement of an anion or the countermovement of a cation via the glucose carrier (38). At the moment, a Na⁺/\( \delta \)glucose stoichiometric ratio of 2:1 is a real possibility (31). Na⁺ binds to the glucose carrier of the intestinal brush border and induces a rapid conformation change in the transporter which increases its affinity for glucose (39,40).

The active transport of glucose is significantly greater in the jejunum than in the mid-ileum, whereas the terminal ileum does not exhibit Na⁺-dependent \( \delta \)glucose transport (41). It has also been shown that the Na⁺-glucose cotransporter activity decreases with age (42). In the rat, fish oil as compared to the butter fat dietary regimen increases the \( n-3 \) fatty acids of the brush border membrane, decreases lipid fluidity, and concomitantly, increases Na⁺-dependent \( \delta \)glucose transport (43).

The biochemical studies on the intestinal mucosa from patients with glucose-galactose malabsorption are in agreement with a defect at the level of the brush border sodium-dependent \( \delta \)glucose transporter. This information has been obtained by different techniques: quantitative radioautography of sugar transport in intestinal biopsies (44), Na⁺-dependent accumulation of glucose in intact small intestinal biopsy specimens (45) (see Table 1), sodium-dependent glucose transport in jejunal brush border membrane vesicles (46), and the measurement of short-circuit current as a function of \( \delta \)glucose concentration in the bathing solution (47,48). One out of four patients with glucose-galactose malabsorption was reported to have no mutarotase activity in the intestinal mucosa (49). More observations are needed before this finding can be interpreted.

**GENETIC CONTROL OF THE GLUCOSE CARRIER**

From the study of the pedigree of six cases, Meeuwisse and Melin were able to conclude that glucose-galactose malabsorption is an autosomal recessive metabolic disorder (50). Recently, Hediger et al. were able to localize the human intestinal Na⁺-glucose cotransporter gene at the \( q 11.2 \rightarrow q \)ter region of chromosome 22; the genes for facilitated glucose carriers, on the other hand, have been mapped to chromosomes 1 and 3 (51). As the Na⁺-glucose cotransporter makes up less than 0.2% of the membrane proteins, Hediger et al. preferred to clone and sequence the gene (52). RNA synthesized from the clone and injected into *Xenopus laevis* oocytes increased Na⁺-dependent sugar uptake more than 1000-fold, and had no effect on the rate of Na⁺-independent uptake. The isolated DNA sequence codes for 662 amino acid residues with a relative molecular mass of 73,080, which is consistent with the Mr of the brush border Na⁺-glucose cotransporter (75,000). The proposed model contains 11 membrane-spanning sequences, and the Na⁺-active site seems to be 30–40 \( \AA \) away from the glucose site. Furthermore, there is no detectable homology between the Na⁺-glucose cotransporter and either the facilitated glucose carrier or the *E. coli* sugar transporters. This suggests that the mammalian Na⁺-driven glucose transporter has no evolutionary relationship to the other sugar transporters (52).
CONCLUSION

In the last 30 years major contributions to the active intestinal transport of glucose and its genetic control have been achieved. The study of patients suffering from glucose-galactose malabsorption has greatly stimulated basic research in the field. On the other hand, comprehension of the function of the glucose-\(Na^+\) transport system greatly changed our insights in the understanding and treatment of acute diarrhea. It is hoped that in the near future the same advances will be obtained in the elucidation of the mechanisms of fructose absorption.

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REFERENCES

DISCUSSION

**Dr. Brodehl:** Tubular glucose reabsorption is also defective in glucose-galactose malabsorption. Do you know whether this is controlled by the same genetic system? Are the renal and intestinal glucose carriers comparable?

**Dr. Eggermont:** This has been suggested, but the information we have for the moment is only clinical evidence and there are no biochemical data that the renal and intestinal glucose carriers are related to each other. The glucose-galactose carrier has also recently been described in the fetal lung. Up to now, we are not aware of clinical manifestations during fetal life in children affected by glucose-galactose intolerance.

**Dr. Hobbs:** If you infuse glucose in your patients with a defective carrier do you get glycosuria?

**Dr. Eggermont:** Yes, and the more glucose you infuse, the more pronounced is the glycosuria.

**Dr. Schaub:** Concerning the discussion on fructose intolerance I want to ask if there are any side effects if fructose is the sole carbohydrate in the diet of these children.

**Dr. Eggermont:** I think fructose in the diet must be limited; otherwise, the child develops osmotic diarrhea. On the other hand, the simultaneous administration of glucose enhances fructose absorption.

**Dr. Schaub:** Is fructose phosphorylated in the gut?

**Dr. Eggermont:** Yes, Ginsburg & Hers (1) showed that fructose is phosphorylated in the small intestinal mucosa as well as in the liver.

**Dr. Schaub:** So the osmotic effect cannot be high.

**Dr. Eggermont:** No, the osmotic effect within the gut lumen is high because you have a system of dose-dependent resorption; on the other hand, active transport is the ideal system for efficient resorption at low concentrations.

**Dr. Baelocher:** You said that in your experience monosaccharide intolerance is no more of any clinical importance, since it has disappeared in recent years. What is your explanation for this? Personally, I have the impression that there are still many discussions about secondary monosaccharide intolerance in clinical practice.

**Dr. Eggermont:** My opinion is very clear. In the past we have exaggerated the problem of sugar intolerance. The best argument for this is that glucose is now used in oral rehydration solutions for treatment of acute diarrhea in many countries.
Dr. Vis: Glucose-electrolyte solutions remain effective, even in case of jejunal atrophy, such as in extreme cases of cow’s milk protein intolerance. Thus the glucose-coupled sodium transport still occurs in these circumstances. Do we know where this transport takes place? Where do we locate the carriers? Over whole of the mucosal surface or only in the crypts?

Dr. Eggermont: The glucose-galactose carrier is localized in the brush border membrane but we have no information where the maximum activity of the carrier is localized, whether on the top or at the base of the villus.

Dr. Vis: How does chloride absorption occur? Where are the chloride transport mechanisms located?

Dr. Eggermont: There is no combined transport of sodium, glucose and chloride. Chloride is absorbed by an independent system.

Dr. Saudubray: Do you know how the transcription of the gene is regulated? Is it regulated by glucose-galactose itself?

Dr. Eggermont: At present we don’t know. It is amazing, however, that this important carrier in the brush border makes up only about 0.2% of the total protein content. In comparison, the sucrase-isomaltase enzyme makes up about 25% of the total proteins of the brush border membrane.

Dr. Hobb: We used to do experiments with yeasts, where if you cultured them in a glucose medium free of galactose they became very good at taking up and utilizing glucose but very bad at taking up galactose. If you then cultured them in a galactose medium free of glucose, exactly the opposite occurred. From these adaptation experiments it seems likely that in the yeasts there are distinctive carriers for glucose and galactose, rather than a common glucose-galactose carrier. Do you know if this has been studied?

Dr. Eggermont: Yes, certainly; it is well known that the facilitative glucose transporters are different from the Na+/glucose cotransporter. More information can be found in the paper by Gould and Bell (2).

REFERENCES