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Intestinal Absorption of Hexoses and Amino Acids: From Apical Cytosol to Villus Capillaries

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Foreword

It is an honor to be invited to contribute a paper to this issue of Journal of Membrane Biology, commemorating the life of Hans Ussing. I only met Hans from time to lucky time at international congresses or at those wonderful, informal and productive Benzon Conferences (Capillary Permeability, 1969; Ion Homeostasis in Brain, 1970; Leaky Epithelia, 1990). Long before these, however, we corresponded about the relation of diffusive and hydrodynamic permeabilities to dimensions of intercellular channels. His 1953 paper on this topic with Koefoed-Johansen [13] was more general and more elegant than the theory published in 1951 by my group that was directed specifically to permeability and pore size of capillary endothelium [18]. In 1952 we also corresponded about the theory of restricted diffusion in relation to Staverman's [27] osmotic reflexion coefficient.
My contribution to this commemorative volume is an original essay; it is presented informally in much the same way as I might have offered it at a Benzon Conference in the Royal Danish Academy with Hans Ussing there to keep everyone on their mettle, trying to meet his rigorous scientific standards.

Introduction

Free hexoses and amino acids generated by membrane-bound hydrolases in the brush border are carried to villus capillary plasma through transcellular and paracellular pathways. The initial steps of carrier-mediated, concentrative transport through apical membranes have been investigated in detail, but relatively little attention has been given to transport from apical cytoplasm to capillaries in the lamina propria of the villus, some 20–30 μm distant in hamster or rat jejunum [1, 2, 15] and 50–60 μm in human jejunum [16]. In the present paper I shall be concerned with this last leg of the overall absorptive process, utilizing glucose as an illustrative example. The hypothesis I will present includes an explanation of the little known (and perhaps controversial) fact that absorptive cells shrink during the absorptive process. For purpose of exposition I will first present the hypothesis in the form of qualitative propositions and subsequently will present quantitative anatomical and functional evidence in support of the propositions.

Propositions: Some Well-established, Some Controversial, Some Novel

(1) Glucose generated from hydrolysis of saccharides in the brush border is concentrated in apical cytosol by the well known SLGT-1 Na-coupled carrier; under some conditions it may also reach apical cytoplasm by facilitated diffusion on Glut-2 carrier that is mobilized by protein kinase C as described by Kellett [11]. Na-coupled transport also induces phosphorylation of light-chain myosin, contraction of the perijunctional actomyosin ring and generalized contraction of the cytoskeleton [15, 17], thereby opening tight junctions and contributing to cell shrinkage as described in Proposition 4 and illustrated in Fig. 1.
(2) Glucose is discharged to post-junctional intercellular fluid from its enhanced concentration in apical cytoplasm, thus providing an osmotic force for fluid absorption through the dilated junctions. This lateral transport step is mediated by facilitated diffusion on the Glut-2 carrier (for review *see* Cheeseman [3] or Kellett [11]).
(3) Fluid absorption through junctions and intercellular channels takes place at relatively high velocity owing to their small cross-sectional areas, particularly in apical regions where lateral membranes of adjoining cells are bonded by tight junctions, maculae adherens and desmosomes.
(4) Intracellular pathways for diffusion of glucose from apical to basal cytoplasm are obstructed by the

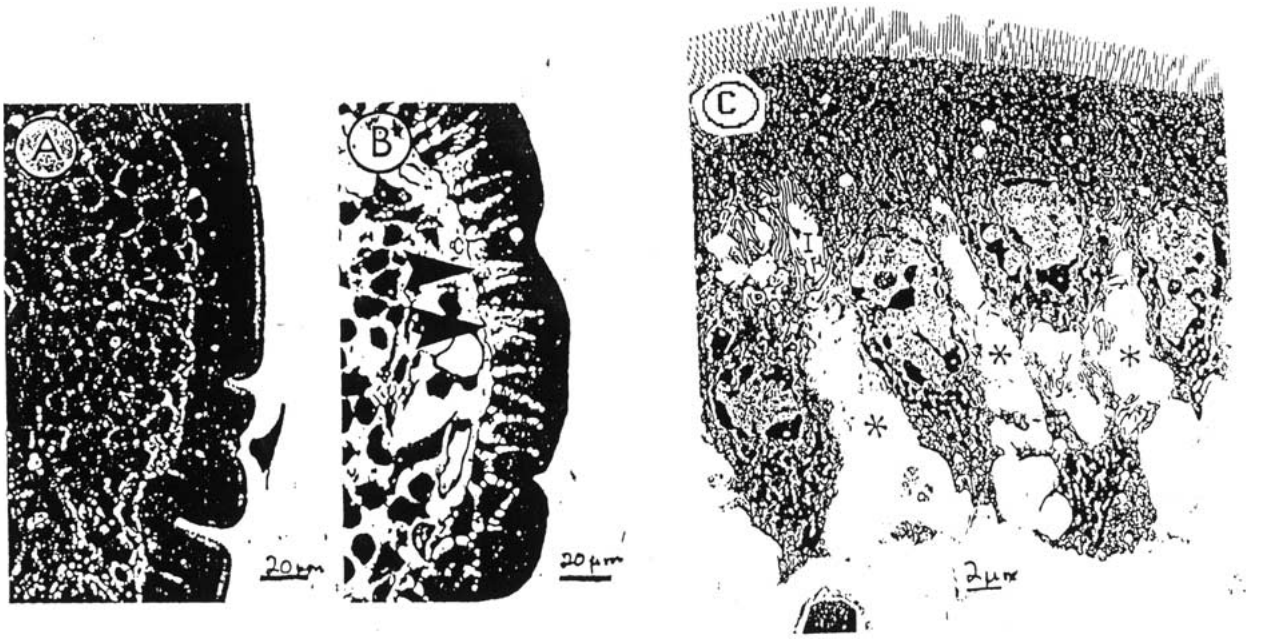

Fig. 1. Light micrographs (*A* and *B*) and lower power EM(*C*) of villus epithelium from hamster jejunum. Rapid fixation by sudden addition of glutaraldehyde during in vitro perfusion with oxygenated fluorocarbon-salt solutions, 38°C. (*A*) Without organic substrate (non-absorbing state). (*B & C*) With 25 mM glucose (absorbing state). Note shrinking of enterocytes with associated widening of intercellular channels during absorption of glucose. Similar changes are found after rapid fixation of segments during perfusion in vivo (*see* Figure 2*C* and *D* of Ref. [15]). The enlarged intercellular spaces allow paracellular passage of both fluid and solutes; in contrast, transport through the cytoplasm is obstructured by the nucleus, mitochondria, endoplasmic reticulum and other membranous structures that occupy much of the volume and cross-sectional area of the enterocytes as shown in Fig. 1*C* or in more detail in references [1, 2, 16]. Fig. 1 *A, B, C* enlarged from Figs. 2 *A* and *B* and Fig. 4 in Madara & Pappenheimer, 1987 [15] (reproduced with permission from *J. Membrane Biol.* 100:149–164).

nucleus and other membranous structures; diffusion is slow compared to rapid transit of concentrated glucose by convection in narrow intercellular channels. Transiently, the high concentration of glucose in lateral channels exceeds the intracellular concentration over most of the lateral surface below tight junctions. Fluid is thus withdrawn from the cells while glucose diffuses from lateral channels into basal regions of the cytosol via Glut-2. Eventually, (1–2 minutes?) equilibration of glucose and a new steady state are reached across lateral membranes, but the cells have lost fluid and the basal portions shrink to a truncated conical base with small cross-sectional area. (5) The high concentrations of glucose brought to basal intercellular spaces by convection are essential for driving the transcapillary flux, which may be 100 × greater per gram tissue than in brain or in skeletal muscles during maximal aerobic exercise.

(6) The lost cellular volume is not recovered until apical transport has ceased, at which time the process outlined in Proposition 4 is reversed until normal cell volume and shape have been restored. This process is rapid; when segments of absorbing jejunum are excised and split longitudinally prior to fixation (a conventional procedure that takes one or more minutes) the cells have already regained their original volume and appear to be in the non-absorbing state [15].

Anatomical Evidence

Figures 1*A*, *B* and *C* (reproduced from Figures 2*A*, *B* and 4 of Madara & Pappenheimer [15] show sections of villus epithelium from segments of hamster jejunum. The tissues were fixed very rapidly by sudden perfusion of the segments with glutaraldehyde at 38°C. In Fig. 1*A* the segments had been perfused in vitro with oxygenated fluorocarbon-salt solution without organic substrates (i.e., in the non-absorbing state). In Figs. 1*B* and 1*C* the segments were fixed under the same conditions as in 1*A* but after perfusion with 25 mM D-glucose (i.e., in the absorbing state). The fluorocarbon is necessary to provide sufficient oxygen for oxidative phosphorylation associated with steady-state Na-coupled transport and regulation of junctions during in vitro perfusions [17, 21].

It is obvious by simple inspection that the absorbing cells are greatly shrunken, leaving widened intercellular spaces, especially in the basal 3/4 of the cells. An estimate of cell volumes under the two

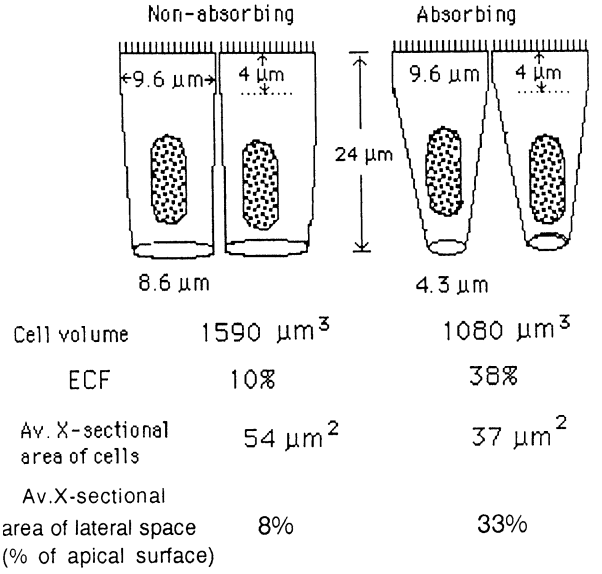

Fig. 2 Dimensions of cells and intercellular spaces as estimated from Figure 1*A* & *B*. The volume of each cell is calculated as an inverted truncated cone of height 20 μm set on a cylindrical base of height 4 μm and radius $r_1 = 4.8$ μm. The secondary radii (r_2) are 4.3 μm in the non-absorbing state and 2.15 μm in the absorbing state. Thus the volumes per cell are $V_{cell} = 4\pi(r_1)^2 + 6.67\pi[(r_1)^2 + r_1r_2 + (r_2)^2]$ or 1592 μm³ in the non-absorbing state and 1080 μm³ during absorption of glucose. The volume of non-absorbing cells calculated as above (i.e., 1592 μm³) is close to that estimated for the fasting hamster by Buschmann & Manke [1] who used the more exact stereological morphometric methods of Weibel & Bolender [28]. The volume of intercellular space (*ecf*) is $V_{ecf} = 24\pi(r_1)^2 - V_{cell}$ or 8% of mucosal volume in the non-absorbing state and 33% during absorption of glucose.

conditions can be made by considering each cell to be in the form of an inverted truncated cone. Figure 2 shows the estimated dimensions of sections of such cells, together with their calculated volumes. The average volume of each enterocyte calculated from the dimensions of Fig. 2 is 1590 μm³ in the non-absorbing state and 1080 μm³ in the absorbing state (i.e, after rapid fixation during perfusion with 25 mM glucose). Although these estimates of volume are admittedly crude, the estimate of volume in the non-absorbing state is approximately the same as that measured by Buschman and Ranke [1] (1452 ± 103 μm³) who used the stereological morphometric techniques of Weibel and Bolender [29]. This agreement for the case of non-absorbing enterocytes lends confidence to our estimates of volume of absorbing cells and to the conclusion that the absorptive mechanism is associated with loss of some 30% of cell volume. As shown in Figs. 1 and 2, the reduction of volume of absorptive cells is matched by a widening of lateral spaces, except in the apical region where adjacent cells are tethered by tight junctions, maculae adherens and desmosomes [17]. Results similar to those in Fig. 1 *A* and *B* were also obtained after rapid fixation in vivo (*see* figures 2 *C* and *D* in Ref. [15]).

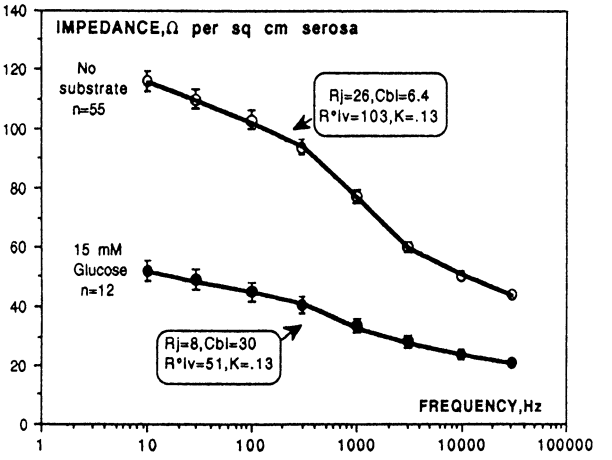

Fig. 3. Effects of glucose on transmucosal impedance of isolated perfused segments of hamster jejunum. The smooth curves passing through the standard error bars are theoretical curves for an electrical analog with components simulating junctional resistance (R_j), resistance of literal spaces plus villus cores (R_{lv}) and capacitance of lateral membranes (C_{bl}). Glucose caused a 3-fold decrease of calculated junctional resistance, a 4.5-fold increase in basolateral membrane capacitance and a 2-fold decrease in resistance of lateral spaces plus villus cores. Reproduced from Ref. [21], with copyright permission from *Am. J. Physiol.*).

Most students of intestinal absorption would predict that enterocytes gain fluid in association with accumulation of glucose during absorption and the anatomical evidence of shrinkage shown in the 1987 paper by Madara and myself (and illustrated in Fig. 1) may be regarded with skepticism. However, strong functional evidence in support of the anatomical evidence comes from measurements of transmucosal impedance during Na-coupled absorption of hexoses and/or amino acids [21].

Functional Evidence

Figure 3, reproduced from Pappenheimer & Volpp [21], shows transepithelial impedances in segments of hamster jejunum perfused with oxygenated fluorocarbon solutions. The variations of impedance with frequency provide measures of junctional resistance (R_j), lateral space resistance (R_{lv}) and lateral membrane capacitance (C_{bl}) as described in Ref. [21]. Perfusion with 15 mM glucose resulted in a decrease in junctional resistance from 26Ω to 8Ω, a decrease in lateral space resistance from 103Ω to 51Ω, and an increase in capacitance from 6 μF to 30 μF (all values expressed per cm² serosa).

The time required to reach a new steady state after addition of glucose was 5–10 minutes, but this includes mixing-time in the perfusion system. Similar changes of impedance are induced by all hexoses and amino acids that are transported through apical membranes by Na-coupled carriers [21] and the magnitude of the

electrical changes are proportional to the transcellular transport rates of the solutes (*see* Fig. 10 of Ref [21]).

The effect of Na-coupled transport on junctional resistance are associated with contraction of perijunctional actomyosin, dilatation of junctions and decrease in number of junctional strands found in electromicrographs of freeze-fractured preparations [15]. The decrease of lateral-space resistance presumably reflects the widening of intercellular channels shown in Fig. 1 and the increase in membrane capacitance presumably reflects the increased exposure of lateral membrane surface.

The results of impedance analysis therefore support the anatomical studies and the proposition that Na-coupled transport of hexoses and amino acids is associated with reduction of cell volume and widening of intercellular channels.

Velocity of Flow in Intercellular Channels

Fluid absorption induced by glucose may reach intercellular channels directly through tight junctions powered by the concentration difference of glucose and sodium across the junctions. Fluid may also reach intercellular channels indirectly through apical membranes in association with Na-glucose cotransport [30] followed by discharge through subjunctional lateral membranes in association with the ATP-Na pump. Flow through the length of the cytoplasm is obstructed by the nucleus, mitochondria and other membranous structures that occupy much of the volume and cross-sectional area of the enterocyte as shown in Fig. 1*C* or in more detail in references [1, 2, 16]. In rats and humans 50–70% of glucose-induced fluid absorption occurs directly through cell junctions as estimated from the clearances of inert solutes from segmental perfusions [20, 24]. It has been estimated that 264 μmols of water pass through apical membranes for each μmol of glucose transported by the human SLGT-1 carrier [30]. Since the transcellular transport maximum for glucose in human jejunum is 170 μmols $hr^{-1}cm^{-2}$ [24], this corresponds to $170\times264\times18\times10^{-6} = 0.8$ ml hr^{-1} cm^{-2} or about 40% of the observed rate of fluid absorption, leaving 60% for the direct path through cell junctions. However, the proportion of fluid absorption reaching subjunctional intercellular channels via direct or indirect pathways is not of importance to the present argument; in either case the *velocity* of flow through any cross-sectional area (a) of intercellular channels is equal to J_v/a, cm t^{-1} where J_v is volume flow rate.

It is obvious from Fig. 1 that the cross-sectional area of intercellular channels increases with distance from the post junctional region near the apex to basal membranes and the velocity will vary inversely with the cross-sectional area. An estimate of the average cross-sectional area of intercellular channels may be made by calculating the average cross-sectional area of cells and subtracting this from the smooth apical surface area. The area so calculated from the dimensions of Fig. 2 is 33% of the apical surface area. In normal 250-gram rats drinking 5% glucose or maltose, the net rate of fluid absorption is about 15 ml hr^{-1} or 0.25 ml hr^{-1} per cm^2 of smooth lumenal jejunal surface [22, 23]; similar values are found in jejunal segments perfused with 50 mM glucose in unanesthetized rats (Table 1). Comparable fluid absorption rates are not available for hamsters, but the dimensions of enterocytes [1, 2] and rates of glucose transport [9, 10] are approximately the same in hamsters as in rats. The velocity of flow through the average cross-sectional area of intercellular channels is therefore about 0.18 cm^3 hr^{-1} $cm^{-2}/0.33$ cm^2 = 0.54 cm hr^{-1} or 1.5 μm sec^{-1}. Given this velocity the transit time required for concentrated glucose to flow from postjunctional to basal regions 20 μm distant would be about 13 seconds. A more sophisticated calculation of mean velocity from the integral of the reciprocal of cross-sectional area as a function of distance (using Simpson's Rule) yields a mean velocity of 1.25 μm sec^{-1} or a transit time of about 16 seconds.

The important point to emphasize is that the transit time of concentrated glucose from apical to basal regions by convection through intercellular channels is less than 20 seconds, whereas diffusion of glucose through the cytoplasm of enterocytes would require at least 2–3 minutes, as discussed in the following section.

Velocity of Diffusion through the Enterocyte

The time (t) taken for a concentration wave front (c/c_o) to travel a distance (x) by linear diffusion can be calculated from Fick's Second Law for any known diffusion coefficient (D). Thus,

$$c/c_o = (1 - 2/\sqrt{\pi} \int_o^y e^{-y^2} dy),$$

where $y = x/2\sqrt{Dt}$ (for references and solutions to this equation *see* Crank: *Mathematics of Diffusion* [4] or D.I. Hitchcock in Chapter 1 of Höber Ref. [8]).

The minimum possible time (t) for 95% of the concentration of glucose at a fixed apical boundary concentration (C_o) to travel 25 μm by free linear diffusion through an unobstructed aqueous solution at 38°C is 75 seconds. In enterocytes, however, the diffusion coefficient in the gelatinous cytosol is less than free and the pathlength for diffusion is circuitous because the cell nucleus, mitochondria and other membranous structures occupy more than 30% of the cell volume [1]. As shown in Fig. 1*C* and in other ultrastructural studies [1, 2, 16] the nucleus alone obstructs most of the cross-sectional area for diffu-

Table 1. Steady-state concentration of glucose in absorbate during perfusion of jejunal segments

	Glucose			
	Mean lumenal mM	J_s μmol/hr/cm^2	J_v ml/hr/cm^2	Absorbate J_s/J_v mM
A. Unanesthetized rats (Calculated from smoothed data of Gromova & Gruzdkov [6])	5	28	0.15	187
	10	40	0.21	190
	20	50	0.26	192
	40	62	0.31	200
	60	70	0.34	206
				195 ± 8 (mean ± SD)
B. Normal humans (Data from many investigators reviewed in ref. [22])	10	115	0.7	164
	20	140	0.9	156
	40	190	1.4	136
	60	227	1.7	134
	80	256	1.8	142
	100	291	1.9	153
				148 ± 12 (mean ± SD)

sion through the cytosol of enterocytes; given the circuitous pathlength and restricted cross-sectional area for diffusion it would be surprising if the time taken for glucose to diffuse from the brush border to the basal membrane were not at least twice the minimum possible time (i.e., >150 seconds). The diffusion coefficient of glucose in 10% gelatine gel is about half that in free solution [5].

The important conclusion is that during the first 1–3 minutes after exposure of the brush border to glucose, the intercellular concentration exceeds the intracellular concentration and thus withdraws fluid from the cell during the time it takes for the glucose to equilibrate across the lateral membranes along the whole length of the cell.

The Steady-State Concentration of Glucose in Fluid Approaching Villus Capillaries

After equilibration of glucose between the rapidly moving intercellular fluid and the cytosol of the enterocyte (i.e., 1–3 minutes after exposure of the apical surface to a constant glucose concentration) a steady state is established, in which the concentration in basal extracellular fluid (i.e., in the absorbate) is approximately equal to the flux per unit fluid absorption (J_s/J_v). Since J_v increases with J_s (until saturation of transporters), it is not surprising to find that J_s/J_v is about constant over a wide range of lumenal glucose concentrations as shown in Table 1. In rats the concentration in absorbate is 195 ± 8 mM and in humans it is 148 ± 12 mM. Presumably, these absorbate concentrations are available for diffusion into capillary plasma as disucssed in the next section.

The high steady-state concentrations of glucose in the absorbates shown in Table 1 (J_s/J_v) are in addition to the electrolytes. The total tissue osmolarity in absorbing regions of the villi (upper 25%) is in the range 400–600 mOsm as determined by depression of freezing point in frozen sections [7]; of this, only 250–300 mOsm is contributed by electrolytes [26].

From Basal Extracellular Fluid (Absorbate) to Villus Capillary Plasma

It seems to have escaped the notice of physiologists that villus capillaries of the jejunum are capable of absorbing glucose at rates (per gram tissue) more than 100× greater than capillaries elsewhere. Thus, 250-gram rats will absorb glucose steadily at the rate of about 5000 μmol hr^{-1} [22] or about 2500 μmol hr^{-1} per gram of jejunal mucosa. Similarly, perfused segments of human jejunum will absorb glucose at the rate of about 400 μmol hr^{-1} cm^{-2} [24] or about 3500 μmol hr^{-1} per gram mucosa. In contrast, transcapillary flux of glucose in brain is less than 30 μmol hr^{-1} per gram brain [12, 19] despite having a carrier for its facilitated diffusion. Even skeletal muscles exercising at 85% of maximum aerobic capacity do not absorb glucose at rates exceeding about 20 μmol hr^{-1} gr^{-1} [28].

There are three possible mechanisms that might account for the fact that intestinal capillaries can absorb glucose 100 times more rapidly per gram tissue than the capillaries of brain or contracting skeletal muscle, namely greater permeability, greater surface area or greater concentration difference across the endothelium. The permeability-surface area product for small lipid-insoluble solutes is 5–10 × greater in villus capillaries than in skeletal muscle [25] but by far the most important factor is the con-

centration gradient. In brain and in working muscle the transcapillary concentration gradients never exceed about 3 mM [19, 28]. In contrast, the concentration of glucose in jejunal absorbate surrounding the villus capillaries is about 150 mM in humans and 195 mM in rats over the entire range of lumenal concentrations shown in Table 1. These high concentrations are brought to the vicinity of capillaries by convective flow of absorbed fluid (solvent drag) as described above. Owing to the large blood flow through the absorbing mucosa (circa 150 ml hr^{-1} g^{-1} [14]), the mean concentration of glucose in villus capillary blood never exceeds about 30 mM. Thus the concentration gradient available for transcapillary absorption is 50–100 fold greater than in brain or contracting skeletal muscle.

Discussion

In this essay I have ventured to speculate on how hexoses or amino acids are transported to villus capillary blood after they have been deposited in high concentration in apical cytoplasm. This part of the absorptive pathway has received little or no attention from physiologists but it is nevertheless an essential part of the overall absorptive process.

The passive component of absorption has often been attributed to "diffusion" but this has little meaning unless the concentration gradients, diffusion coefficients and pathways through the cytosol or through intercellular channels can be specified. The pathway for diffusion of glucose through enterocytes is a circuitous one around the nucleus, mitochondria and other membranous structures; even if the diffusion pathway were unobstructed, the diffusion of glucose from apical to basal cytoplasm would be slow compared to transport by convection in fluid flow through intercellular channels. One important consequence of this disparity is transient osmotic withdrawal of fluid from enterocytes with loss of cell volume and corresponding increase in volume of extracellular space and widening of lateral spaces. A second important consequence is the transport without loss of concentration (i.e., by convection in intercellular channels) to the vicinity of villus capillaries. High concentrations in the vicinity of capillaries achieved by convective transport are essential for transcapillary transport, which must accommodate fluxes more than 100-fold greater than in capillaries elsewhere in the body. The mucosa of the small intestine is less than 0.5% of body weight but its capillaries must absorb intermittent loads of glucose that far exceed the average metabolic demands for glucose by the entire body.

Doubtless, these novel speculations will be controversial, but they are offered at this time to stimulate interest in an aspect of nutrient absorption that has received less than its fair share of attention. I would like to think that Hans Ussing would have approved, though he might have had some incisive and constructive criticisms.

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