© 2017. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2017) 220, 2495-2501 doi:10.1242/jeb.144048

COMMENTARY

Integrative physiology of transcellular and paracellular intestinal absorption

William H. Karasov*

ABSTRACT

Glucose absorption by the small intestine has been studied for nearly a century. Despite extensive knowledge about the identity, functioning and regulation of the relevant transporters, there has been and there remains controversy about how these transporters work in concert to determine the overall epithelial absorption of key nutrients (e.g. sugars, amino acids) over a wide range of dietary and/or luminal concentrations. Our broader, integrative understanding of intestinal absorption requires more than the reductionist dissection of all the components and their elaboration at molecular and genetic levels. This Commentary emphasizes the integration of discrete molecular players and processes (including paracellular absorption) that, in combination, determine the overall epithelial absorption of key nutrients (e.g. sugars, amino acids) and putative anti-nutrients (water-soluble toxins), and the integration of that absorption with other downstream processes related to metabolic demands. It identifies historic key advances, controversies and future research ideas, as well as important perspectives that arise through comparative as well as biomedical physiological research.

KEY WORDS: SGLT1, GLUT2, Paracellular absorption

Introduction

Solute absorption across the intestinal epithelium can be transcellular [i.e. through the intestinal cell (enterocyte), crossing the apical and basolateral membranes] or paracellular (i.e. crossing the epithelium between cells) (Fig. 1). Intestinal glucose absorption has been studied for nearly a century (e.g. McCance and Madders, 1930; Wertheimer, 1934), and the modern view that it is driven predominantly by Na^+-coupled glucose absorption across the apical, or brush border, membrane of enterocytes especially emerged in the three decades beginning in the 1960s (Table 1). In the past three decades, knowledge on the mechanisms of intestinal glucose absorption has continued to advance, partly thanks to the existence of alternative views that include an important paracellular pathway. This Commentary considers some of the historic key advances across the field, and discusses controversies and future research ideas, as well as perspectives that arise through comparative as well as biomedical physiological research.

An integrated view of nutrient absorption

The Na^+-coupled glucose transporter (SGLT1), which mediates transcellular absorption, was sequenced and expression cloned in 1987 by Wright and colleagues (Hediger et al., 1987; Table 1). With the advent of the molecular age, it may have occurred to some students of intestinal membrane physiology that knowledge about absorption would subsequently advance mainly through continued reductionist focus on the molecular details of transporters and their regulation. But, notably, also in 1987, John Pappenheimer suggested there were important overlooked elements that led him to propose an additional complementary process underlying nutrient absorption – a major role for paracellular transport [i.e. transport through the tight junctions (TJs) between enterocytes] of hydrophilic organic solutes by diffusion and/or solvent drag (Madara and Pappenheimer, 1987; Pappenheimer, 1987; Pappenheimer and Reiss, 1987). Since 1987, we have continued to learn more molecular and regulatory details about SGLT1 and other members of its carrier family (Wright et al., 2011), as well as about intestinal transporters in other solute carrier families (Hediger et al., 2004) for sugars [e.g. glucose transporters GLUT2 and GLUT5 in solute carrier (SLC) family 2] and amino acids (e.g. transporters in SLC family 1). But, notwithstanding all this knowledge about transporters, there has been and there remains controversy about how they work in concert to determine overall epithelial sugar and amino acid absorption over a wide range of dietary and/or luminal concentrations. A recent paper (Naftalin, 2014) and corresponding set of published peer reviews and commentaries bears witness to this controversy.

Gaps in knowledge sometimes become especially apparent when changing our frame of reference from the specific reductionist perspective to the more integrated whole-animal view. Pappenheimer was trying to reconcile the very high whole-animal carbohydrate assimilation rates of commonly studied laboratory mammals with his own and others' much lower measurements of maximal mediated absorption rate ($\dot{V}_{max}$) of glucose obtained using isolated tissues or perfused intestines *in situ* (Pappenheimer, 1990, 1993). Likewise, others among us struggle to incorporate carbohydrate digestion and absorption by wild animals, such as nectarivorous birds and bats, into the sugar oxidation cascade, which refers to the path of carbon from flowers through the digestive system all the way into the mitochondria (Suarez et al., 2011). This approach, which has been identified most recently as the vertical integration of physiological processes across organizational levels within organisms, can be considered as one of the grand challenges in physiology (Mykles et al., 2010). Nectarivorous birds and bats are particularly interesting animals in this regard because when they are hovering they have among the highest metabolic rates per unit body mass of any vertebrate, and studies using hovering birds and bats feeding on sugar solutions labeled with stable isotopes have shown that 80–90% of their metabolism is supported directly by assimilated dietary carbohydrate (Suarez et al., 2011). Have these animals been demonstrated to have high enough intestinal glucose $\dot{V}_{max}$ to explain this empirical wonder? The answer is no, and in the course of this Commentary I'll explain why and also how the answer most likely involves paracellular absorption.

Department of Forest and Wildlife Ecology, University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706, USA.

*Author for correspondence (wkarasov@wisc.edu)

W.H.K., 0000-0001-9326-5208

Journal of Experimental Biology (2017) 220, 2495-2501 doi:10.1242/jeb.144048

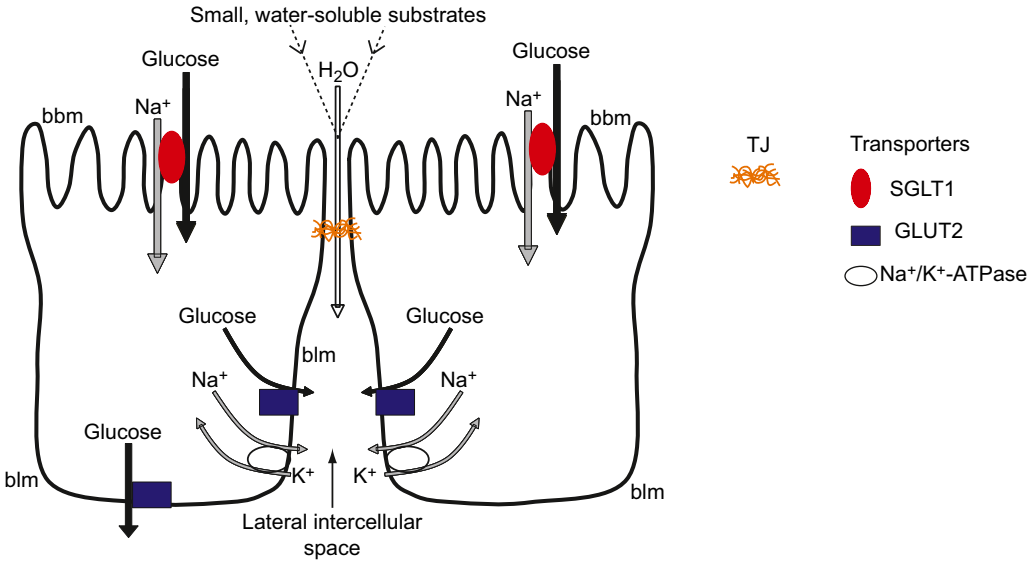

Fig. 1. Schematic diagram illustrating adjacent enterocytes and the mechanisms by which glucose is absorbed. Transcellular absorption: glucose from the lumen (top of the figure) can move across the epithelium by uptake via the Na^+-coupled glucose transporter (SGLT1) in the brush border membrane (bbm), and then exit through the basolateral membrane (blm) via glucose transporter 2 (GLUT2). Na^+ ions, which move down their electrochemical gradient, are expelled from within the cell by Na^+/K^+-ATPase in the basolateral membrane (the exact stoichiometries of solute fluxes are not depicted). Paracellular absorption: glucose can also cross the epithelium between the enterocytes through the tight junction (TJ; composed of a number of interacting protein strands such as claudins and occludin) via diffusion or solvent drag. Solvent drag is bulk movement of solute along with absorbed water, the movement of which is osmotically coupled to solute transport into the lateral intercellular space.

Is paracellular transport important for glucose absorption in mammals?

Pappenheimer directed attention to measurements by him and others of considerable intestinal absorption of small- to medium-sized hydrophilic compounds such as creatinine [molecular weight (MW) 113] and mannitol (MW 182) that do not interact with transporter proteins (Pappenheimer, 1990). Because the phospholipid bilayer of intestinal enterocytes is absorption-limiting for hydrophilic compounds (Diamond and Wright, 1969; Smulders and Wright, 1971), it was presumed that those hydrophilic organic molecules that do permeate across the small intestinal mucosal epithelium do so primarily through the paracellular pathway, a presumption visually supported by autoradiography (Ma et al., 1993) and confocal laser scanning microscopy (Hurni et al., 1993; Chang and Karasov, 2004a). Pappenheimer and colleagues thought that Na^+-coupled glucose or amino acid absorption enhanced the permeation of hydrophilic compounds through the paracellular pathway, by increasing solvent drag as water is absorbed and by altering structural features that influence TJ permeability (Madara and Pappenheimer, 1987; Pappenheimer, 1987; Pappenheimer and Reiss, 1987). Pappenheimer thought that the amount of this enhanced absorption was large relative to that mediated through SGLT1 and that, when combined, the two mechanisms could quantitatively explain glucose absorption in mammals. In a thoughtful review a decade after Pappenheimer's proposals, Ferraris and Diamond (1997) agreed that solvent drag 'secondary to carrier-mediated glucose (and amino acid) transport should yield a further passive component of glucose and amino acid absorption', but they doubted its quantitative importance for glucose absorption in terrestrial mammals. I partly agree, and having considered some of their arguments along with the recent discussion in the literature (Naftalin, 2014), I think there are two findings that in particular blunt Pappenheimer's argument as it applies to most mammals, though one of them is controversial and might even be interpreted to support paracellular absorption. I'll outline these findings, which relate to some of the most current thinking and controversy about intestinal glucose absorption in laboratory mammals, and then outline why, whatever their resolution, there is good reason to think that paracellular absorption is important in certain other animals.

Table 1. Highlights in the advance of knowledge about intestinal glucose absorption

Year	Highlight	Reference
1960	Crane proposes Na^+-coupled glucose absorption across the intestinal brush border membrane	Crane et al., 1961
1970	Concept extended to coupled transport of Na^+ and other organic solutes	Schultz and Curran, 1970
1974, 1980	Description of exit of glucose across basolateral membrane of enterocytes by facilitated diffusion (through transporter later defined as GLUT2)	Murer et al., 1974; Wright et al., 1980
1986	Dietary carbohydrate induction of mediated apical glucose transport caused by induction of glucose transporters	Ferraris and Diamond, 1986
1987	Sequencing and expression cloning of Na^+/glucose co-transporter SGLT1	Hediger et al., 1987
1987	Pappenheimer proposes major role for paracellular transport of organic solutes by diffusion and/or solvent drag	Madara and Pappenheimer, 1987; Pappenheimer, 1987; Pappenheimer and Reiss, 1987
1994	Majority of glucose absorbed by a non-mediated (paracellular) pathway in a nectarivorous bird	Karasov and Cork, 1994
2000	Kellett describes major role of glucose-induced recruitment of GLUT2, which mediates facilitated diffusion of glucose across the brush border membrane	Kellett and Helliwell, 2000

Journal of Experimental Biology

Journal of Experimental Biology (2017) 220, 2495-2501 doi:10.1242/jeb.144048

Countering Pappenheimer: downplaying the paracellular pathway for glucose absorption in terrestrial mammals

The real $\dot{V}_{max}$ for intestinal glucose absorption is often underestimated

Most of our measurements underestimate the real $\dot{V}_{max}$ for intestinal glucose absorption, which leads to overestimation of the proportional contribution of paracellular absorption. Ferraris and Diamond (1997) highlighted measurements made in unanesthetized rats (Uhing and Kimura, 1995a,b; Uhing, 1998) and humans (Fine et al., 1993, 1994, 1995), which is important because anesthesia and surgical intestinal manipulation markedly decrease the rate of mediated glucose absorption (Uhing and Kimura, 1995a). In their work on intestinal absorption under normal physiological conditions, Uhing and Kimura (1995a) used chronically catheterized rats (i.e. animals unaffected by intestine manipulation or anesthesia at the time of measurement). These rats were infused with non-metabolizable 3-*O*-methyl-D-glucose (3OMG) into the duodenum, and its appearance in the blood was measured; L-glucose was used to correct the total absorption for the passive (i.e. non-mediated) component, as it is only passively absorbed. The authors reported a $\dot{V}_{max}$ of 0.93 µmol min^{-1} cm^{-1} along a length of duodenum plus proximal jejunum equal to about 15% of the whole intestine length (Uhing and Kimura, 1995a). This is about 4–5 times higher than that measured during steady-state *in vivo* jejunal perfusion of anesthetized rats by Uhing and Kimura (1995a) (0.18 µmol min^{-1} cm^{-1}) or in a dozen other studies by eight different investigators (mean 0.24 µmol min^{-1} cm^{-1}, range 0.16–0.32; summarized in Pappenheimer, 1990). Even measurements of $\dot{V}_{max}$ made *in vitro* by Diamond and colleagues using everted intestinal sleeves from proximal jejunum of laboratory rats are lower, by 30% (table 3 in Toloza and Diamond, 1992, compared with the $\dot{V}_{max}$ reported by Uhing and Kimura, 1995a).

When the absolute magnitude of $\dot{V}_{max}$ is underestimated, so is its proportional contribution to total glucose absorption; consequently, the proportional contribution of passive absorption is overestimated. So, although Pappenheimer's arguments were motivated by the mismatch between the very high carbohydrate assimilation rates of commonly studied laboratory mammals and his own and others' much lower measurements of $\dot{V}_{max}$, part of the problem was that we routinely underestimate $\dot{V}_{max}$. This brings us to a second finding that may help to resolve the mismatch, though we will first briefly revisit our example of hovering nectarivores.

The highest intestinal $\dot{V}_{max}$ for glucose absorption measured in any vertebrate using everted intestinal sleeves was in hummingbirds – a value of 1.26 µmol min^{-1} summed over the entire intestine nominal area (or >1 µmol min^{-1} cm^{-2} over the most proximal two thirds; Diamond et al., 1986). If we took this to be an underestimate by 50% of the bird's actual capability, would that be enough to account for their apparent rate of sugar assimilation while hovering? No. Their measured hovering metabolic rate (657 J g^{-1} h^{-1}; Suarez et al., 2011) converts to 11.7 µmol sugar min^{-1} [considering their body mass (3.25 g) and 0.33 µmol sugar J^{-1}], of which almost all (95%) comes directly from the diet. You can see that the measured $\dot{V}_{max}$ for glucose absorption, even if adjusted upwards, still accounts for only about 25% of the apparent actual rate of sugar absorption by a hovering hummingbird. Calculations are rather similar for hovering nectarivorous bats (Rodriguez-Peña et al., 2016).

Controversy about the contribution of GLUT2

In a seeming further blow to Pappenheimer's argument, it has been reported that GLUT2, another sugar carrier, may account for a considerable amount of glucose absorption that was perhaps thought to be paracellular. More than a decade after Pappenheimer's provocative proposal about paracellular absorption, and 3 years after the review by Ferraris and Diamond (1997), George Kellett proposed that what previously appeared to be a diffusive component of sugar absorption in the rat, a component assigned by Pappenheimer to be paracellular absorption, was actually sugar absorption mediated by the transporter GLUT2 (Kellett and Helliwell, 2000). Prior to this, GLUT2 had been assigned the role of a low-affinity transporter in the enterocyte basolateral membrane (Fig. 1). Here, GLUT2 was thought to move glucose across the basolateral membrane down its concentration gradient to the blood. Kellett's group provided evidence that high luminal glucose concentration induced the activation and recruitment of GLUT2 to the apical or brush border membrane, where it could facilitate glucose and fructose entry into the enterocyte at high luminal sugar concentrations at rates even faster than SGLT1 when the latter is saturated. Because GLUT2 is a low-affinity carrier, glucose absorption rate via this route increases rather linearly with increasing glucose concentration, giving the appearance of passive glucose absorption. Kellett et al. (2008) described a putative mechanism for GLUT2 recruitment to the brush border membrane that was activated by glucose transport through SGLT1.

Elements of this work have been challenged, however. Wright et al. (2011) thought that a flaw in the findings of Kellett and Helliwell (2000) was due to their reliance on phloretin, which is an inhibitor of GLUT2, to dissect the contributions of SGLT1 and GLUT2 to glucose absorption in rats. Wright et al. (2011) pointed out that phloretin is also a potent non-competitive inhibitor of SGLT1, and so Kellett and Helliwell (2000) underestimated the importance of SGLT1 and thus overestimated the importance of GLUT2. Indeed, subsequent work on both transporters, using wild-type mice and mouse transporter knockouts, indicated that after a high glucose load either some GLUT2 (Gorboulev et al., 2012) or no GLUT2 (Roder et al., 2014) was incorporated into the brush border membrane, but in either case GLUT2 had little or no impact on glucose absorption in comparison to SGLT1. But, as is revealed in the debate related to Naftalin (2014), and even stated in Roder et al. (2014), the different findings about the role of GLUT2 in glucose absorption might result from particular diet effects or methods or even species differences, and the controversy may continue.

Additionally, the interesting work of Naftalin (2014) suggested that the presence of GLUT2 in the apical membrane might reduce transcellular glucose absorption powered by SGLT1, and actually lead to increased paracellular flux of glucose. This idea is supported by computer modeling, which suggests that, during concentrative glucose transport by SGLT1, the presence of GLUT2 in the brush border membrane would lead to the efflux of glucose via GLUT2 back to the lumen, essentially reducing transcellular glucose transport. The glucose that effluxes back across the brush border membrane via GLUT2 to the lumen would cross the epithelium through the paracellular route. The model is fascinatingly intricate in its detail, with obviously an interesting and provocative output. But, that also makes it a challenging proposal for others to evaluate without installing the computer model, vetting its various assumptions and delineating experimental tests of its veracity. Space here does not permit us, nor is it the goal of this Commentary, to consider this issue or to attempt to resolve the controversy about the role of GLUT2. For now, this example is offered as an illustration of how new – even controversial – ideas continue to come forward, and how this continuing process stimulates our

thinking about new research. Just as two examples, reading the back-and-forth discussion of the model proposed by Naftalin (2014) revealed to me that (i) no one, to my knowledge, has demonstrated the apical presence of GLUT2 in avian intestine (which is relevant to our discussion of glucose absorption in hummingbirds), and (ii) the primary role of GLUT2 in the basolateral membrane as the efflux route for glucose from enterocytes is even debatable and there may be other routes of glucose efflux to discover. This latter point is supported by studies with mice and humans in which GLUT2 is knocked out or congenitally absent, respectively; in these cases, glucose absorption does not appear to be impaired (Wright et al., 2003).

Notwithstanding these unresolved and controversial mechanisms of intestinal glucose absorption, which include paracellular absorption and GLUT2 in the brush border membrane, the primary role of SGLT1 seems established. The model proposed by Crane (Crane et al., 1961) for active uptake of glucose by intestinal enterocytes remains valid today (Wright et al., 2011), and it seems to account for the majority of glucose absorbed by laboratory mammals. Putative additional absorption mechanisms rely on uptake by SGLT1 as a necessary first step: it may activate the mechanism for GLUT2 recruitment (Kellett et al., 2008) and stimulate alterations in structural features that influence TJ permeability (Madara and Pappenheimer, 1987), and the SGLT1-linked solute flux creates a force for paracellular absorption by solvent drag through the TJs (Pappenheimer, 1987; Pappenheimer and Reiss, 1987). The primary role of SGLT1 is underscored by the fact that SGLT1-knockout mice (SGLT1$^{-/-}$) have impaired glucose absorption and die within 2 days of weaning when fed standard carbohydrate-containing diet, but survive if fed a diet that does not contain monosaccharides and disaccharides (Gorboulev et al., 2012). The picture is similar in human neonates with mutations in SGLT1, which causes glucose–galactose malabsorption (Wright et al., 2003). In contrast, GLUT2-null mice, and patients with GLUT2 deficiency, do not exhibit such extensive impairment of glucose absorption (Wright et al., 2003; Roder et al., 2014).

Considering the findings regarding the primacy of SGLT1 in glucose absorption in laboratory mammals, does this mark the end of further consideration of paracellular absorption as a major component of intestinal absorption of hydrophilic organic solutes including glucose and amino acids? I do not think that this is the case for reasons discussed in more detail below.

Arguing the case for paracellular absorption

The role of the paracellular pathway in non-laboratory animals

There is extensive evidence of a quantitatively important paracellular pathway in other kinds of organisms besides laboratory mammals. Cases in point are small birds and bats. To measure paracellular absorption, one can use some of the many hydrophilic, metabolically inert, organic molecules that do not interact with intestinal transporters and are in the MW size range of amino acids and sugars (approximately 75–180 Da). In our work on sugar absorption, we have used radiolabeled L-glucose (MW=180.6), if possible, or L-arabinose (MW=150.1) and L-rhamnose (MW=164.2) (Caviedes-Vidal et al., 2007; Price et al., 2015). We have validated all of these molecules as probes of passive absorption in birds, bats and non-flying mammals (Lavin et al., 2007; Price et al., 2014). Because these probes do not interact with intestinal transporters, if they are orally administered to intact animals and then appear in blood or urine, this is evidence of paracellular absorption somewhere along the gastrointestinal tract. One can then use simple pharmacokinetic models to calculate the fractional absorption, f (sometimes called bioavailability), values of which range between 0 (no absorption) and 1 (complete absorption). Theoretical reasons why differences in f between species should correspond to differences in permeability per unit area of intestine are discussed elsewhere (Chediack et al., 2003).

By combining our data with those of others in the literature (across a total of 23 species), we have found that f in flying bats and birds averages 0.74±0.05 (mean±s.e.m.), which is 3 times higher than that averaged across 19 species of terrestrial mammals (0.25±0.03) (Price et al., 2015). Differences of this magnitude between flyers and non-flyers hold up if one controls statistically for effects of molecule size on f (Karasov, 2011) or if one makes the comparison for individual molecular probes (Price et al., 2015) or whether or not measurements were made in the presence of luminal nutrients (our typical protocol) (Caviedes-Vidal et al., 2007). The difference between flyers and non-flyers in small intestinal permeability to these probes is possibly even larger than indicated in the whole-animal experiments, because among the terrestrial mammals some probe may have been absorbed in the large intestine, cecum or colon (e.g. Yuasa et al., 1997), whereas most of the birds and bats we studied have only a small intestine. When we anesthetized seven species of bats and five species of rodents and measured the clearance of the probe molecules per cm^2 of nominal area in segments of small intestine *in situ*, clearance of the probe in the bats exceeded that in the rodents by 4.7 times (Price et al., 2015).

Both the whole-animal and *in situ* measurements indicate that bats and birds have much higher small intestinal permeability to hydrophilic organic solutes than do terrestrial mammals. Could this enhanced paracellular pathway account for the majority of glucose absorption? In order to determine this, we can use the 'gold standard' method of assessing significance of paracellular permeability favored by Ferraris and Diamond (1997) in their review. This method compares the actual rates of appearance in the blood of non-metabolizable 3OMG and of passive permeability probes; these rates are measured simultaneously in the same animals. In our experiments, after the two kinds of probes are administered orally, the fractions absorbed into blood typically rise rapidly to a plateau in 60–100 min for both probes; there is no particular long lag for the paracellular probes (see examples for six species in Fig. 2). Apparent rates of absorption of the paracellular probes (based on linear slopes for early sampling time points <25 min) in the two non-flyers (Fig. 2C,D), relative to total 3OMG absorption that was measured simultaneously over the identical interval in each species, averaged 0.31±0.14. This value was likely an overestimate because the paracellular probe molecules that were used were smaller in molecular size than glucose, and smaller molecules cross the TJ faster (Karasov, 2011). Thus, the results with the rats and marmosets indicate that the majority of glucose was absorbed by mediated pathway(s), as was found previously by Uhing and Kimura (1995b) in rats and by Fine et al. (1993, 1994, 1995) in a different primate – humans. In contrast, the same ratio in the four flyers depicted in Fig. 2A,B,E,F was significantly higher (P=0.037), averaging 0.81±0.13, meaning that the majority of glucose was absorbed passively in the small birds and bats.

Recall that in our example of the hummingbird drinking nectar while hovering, the measure of $\dot{V}_{max}$ for glucose absorption, even when corrected upwards for technical reasons, could account for only about 25% of the very high rate of absorption actually achieved by hovering hummingbirds. It is uncertain whether there is a need to include in our calculation an additional component of mediated absorption owing to the presence of GLUT2, because this has never

Journal of Experimental Biology

Journal of Experimental Biology (2017) 220, 2495-2501 doi:10.1242/jeb.144048

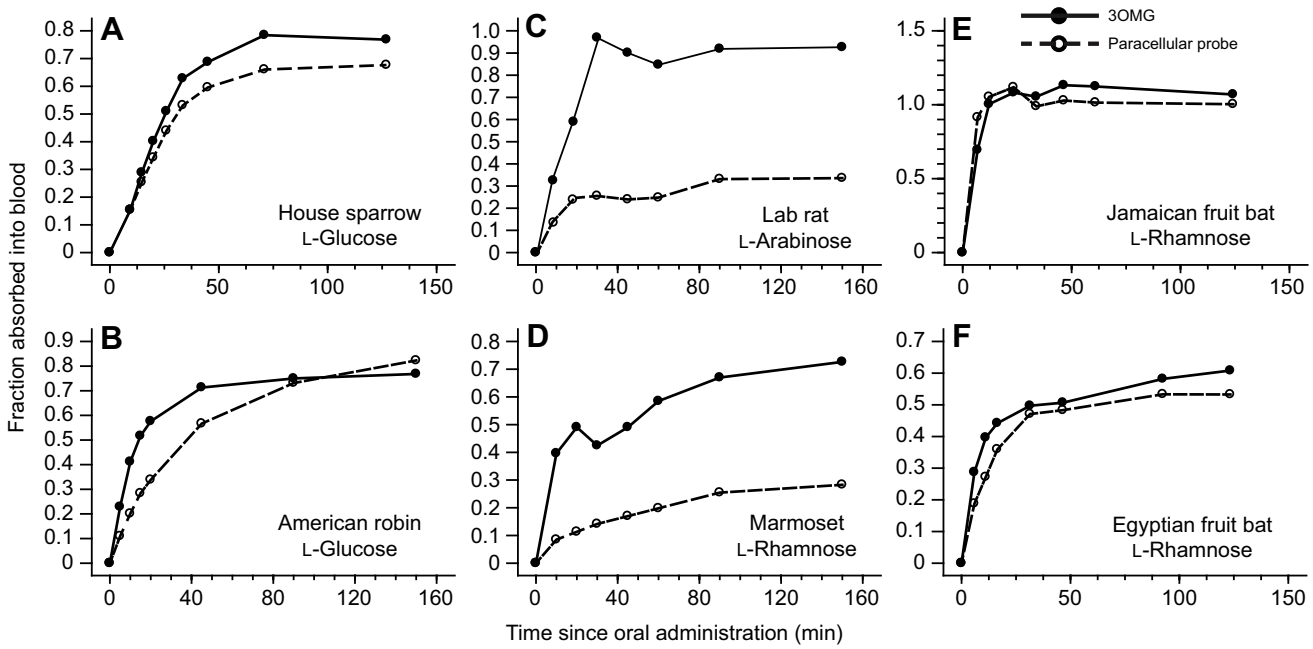

Fig. 2. Cumulative absorption of 3-*O*-methyl-D-glucose (3OMG) and a paracellular probe as a function of time since oral administration. The figure shows four species of flying vertebrates (birds: A,B; bats: E,F) and two non-flying mammals (C,D). The common name of the species and the paracellular probe that was administered are given in the lower right of each panel. The glucose analog and the paracellular probe (both non-metabolizable) were administered simultaneously to non-fasted individuals, and the *y*-axis is the fraction of dose administered that was absorbed. Mean values for each study population (typically at least 6 individuals/species) are shown. Details of the experiments, and the analytical methods, are found in the following sources: A, Chang and Karasov, 2004b; B, McWhorter et al., 2010; C, Lavin et al., 2007; D, McWhorter and Karasov, 2007; E, Caviedes-Vidal et al., 2008; F, Tracy et al., 2007.

been tested for in any bird. But, in any event, the above analysis of paracellular absorption, which includes a measurement made in intact feeding hummingbirds using L-glucose (McWhorter et al., 2006), seems consistent with the idea that the majority of glucose absorption could have been passive.

Why do birds and bats have more permeable intestines than non-flying mammals, allowing more passive paracellular absorption? Small birds and bats generally have shorter small intestines with less nominal surface area than similarly sized non-flying mammals, probably because they evolved under selective pressure to reduce the size of the gut and the mass of the digesta it carries (Caviedes-Vidal et al., 2007; Price et al., 2015). The high rate of passive, paracellular absorption of amino acid- and glucose-sized molecules per cm^2 of intestine in flying animals could compensate for any reduction in transporter-mediated absorption associated with their smaller intestines.

Paracellular absorption: beyond glucose transport

When we restrict our consideration of paracellular absorption to the narrow issue of glucose absorption, we may miss important research opportunities. The field's near-solitary focus on the issue of whether most glucose is absorbed actively or passively fails to confront the empirical reality that there are pathway(s) for absorption of other small- to medium-sized hydrophilic molecules along the gastrointestinal tract. Based on our literature review (Caviedes-Vidal et al., 2007), measurements of f using the passive probes L-arabinose, L-rhamnose and mannitol average 0.14±0.05 across eight species of intact domesticated animals and 0.15±0.06 across all three probes in humans. Because of the kinetics of non-mediated absorption, this means that 15% of any dose or concentration of these or similar kinds of compounds is potentially absorbed (Chediack et al., 2001). Because of the size-sieving effect of TJs (Chediack et al., 2003), the rate and extent of absorption is inversely related to the molecular size of the hydrophilic probes, and so f for a smaller molecule like creatinine is even higher. Indeed, in intact animals, the majority of orally dosed creatinine (MW 113), a paracellular probe comparable in size to the amino acid proline (MW 115), is absorbed in rodents, bats, humans and other mammals (Price et al., 2015). Hence, the paracellular pathway is arguably a non-trivial pathway for the absorption of other hydrophilic molecules besides glucose, such as amino acids, naturally occurring secondary metabolites in foods (Karasov, 2011), anthropogenic toxicants and drugs. Related to this, there are a number of lines of research on paracellular absorption worth highlighting because of their biomedical significance, though it is not the intent of this Commentary to review them. Many intestinal pathologies involve changes in paracellular absorption (e.g. see table 1 in Krause et al., 2008) and consequently there have been decades of research on the best ways to clinically measure it (e.g. van Wijck et al., 2013). There are active bodies of research on the best models for predicting paracellular drug absorption (e.g. Da Silva et al., 2015) and on the enhancement of paracellular absorption of hydrophilic drugs (e.g. Dittmann et al., 2014; Zhu et al., 2014).

There may yet be other species with relatively high rates of paracellular absorption. Among the terrestrial mammals, there appear to be significant differences in f among species (He et al., 1998). In comparing, for example, many measures of f in humans and rats by different laboratories, one finds that f in humans for orally administered mannitol is about 7 times greater than that in rats (Chediack et al., 2003); this correlates with the fact that the oral bioavailability of water-soluble drugs is greater in humans than in rats (He et al., 1998). Paracellular absorption in poultry, on whom we rely for a food source, should certainly be checked. Ferraris and

Diamond (1997) suggested that intestinal passive permeability to glucose and amino acids may be higher in seawater- compared with freshwater-adapted fish (see also Gunzel and Yu, 2013). In insects, the functional features of the paracellular pathway across the midgut epithelial monolayer are largely unknown, but the available data indicate that, as in vertebrates, it is selective with respect to the size and charge of molecules and its permeability can be modulated (Fiandra et al., 2009). This has made the paracellular pathway one focus of research on biopesticide absorption as a strategy for pest control (Fiandra et al., 2009).

Conclusions

Our knowledge is scanty about the mechanisms that underlie differences in paracellular absorption between species (Price et al., 2015). Claudin proteins, which are a large family of membrane-spanning proteins that modulate paracellular permeability, were only discovered in 1998 (Lingaraju et al., 2015). Though we are beginning to understand the roles of individual claudins, some of which are thought to form charge- and size-selective TJ pores for smaller molecules, we know relatively little about how they interact with each other and with other better-known TJ proteins such as occludin or ZO-1 (Gunzel and Yu, 2013). Those latter proteins may play more of a role in influencing TJ permeability to macromolecules (Lingaraju et al., 2015). Presumably, we will one day understand the differences in intestinal paracellular permeability between flying and non-flying vertebrates in terms of these proteins and their interactions. But, also, in accordance with August Krogh's dictum (Krogh, 1929), the study of the cellular and subcellular details of paracellular absorption in general might be advanced by the study of species such as birds and bats with relatively high paracellular absorption.

The historical elements of this Commentary bring home two final general points about research opportunity. Pappenheimer's original proposal of a major role for paracellular transport in nutrient absorption stimulated a lot of important research and arguably led to unanticipated discoveries such as the findings on GLUT2 trafficking and a major difference in intestinal absorption between flying and non-flying vertebrates. There may well be more to come. The second point is apt in our genomic age of biology – our understanding of intestinal absorption advances by both the reductionist delineation of all the components involved and the integration of their function in relation to other downstream processes related to overall metabolic demands in diverse species. The view that there is important biology beyond genes was beautifully articulated in The Music of Life (Noble, 2006) for the case of the heart's rhythm, but it is a lesson worth teaching for most areas in physiology, including intestinal absorption.

Acknowledgements

I thank the editors for the invitation to prepare this commentary. My work in this area was in collaboration with many colleagues who are also dear friends, but especially Dr Enrique Caviedes-Vidal. I dedicate the piece to Dr Jared Diamond, and to Dr John Pappenheimer (now deceased), two influential workers on paracellular permeability and on intestinal glucose transport whom I have had the pleasure to know and who were especially influential in my learning about intestinal physiology. However, the perspectives offered are entirely my own. I thank the editors and reviewers for many constructive comments for improving the manuscript.

Competing interests

The author declares no competing or financial interests.

Funding

Financial support came most notably from the National Science Foundation and the Department of Forest and Wildlife Ecology at University of Wisconsin-Madison.

References

Caviedes-Vidal, E., McWhorter, T. J., Lavin, S. R., Chediack, J. G., Tracy, C. R. and Karasov, W. H. (2007). The digestive adaptation of flying vertebrates: high intestinal paracellular absorption compensates for smaller guts. *Proc. Natl. Acad. Sci. USA* **104**, 19132-19137.

Caviedes-Vidal, E., Karasov, W. H., Chediack, J. G., Fasulo, V., Cruz-Neto, A. P. and Otani, L. (2008). Paracellular absorption: a bat breaks the mammal paradigm. *PLoS ONE* **3**, e1425.

Chang, M.-H. and Karasov, W. H. (2004a). Absorption and paracellular visualization of fluorescein, a hydrosoluble probe, in intact house sparrows (*Passer domesticus*). *Zoology* **107**, 121-133.

Chang, M.-H. and Karasov, W. H. (2004b). How the house sparrow, *Passer domesticus*, absorbs glucose. *J. Exp. Biol.* **207**, 3109-3121.

Chediack, J. G., Caviedes-Vidal, E., Karasov, W. H. and Pestchanker, M. (2001). Passive absorption of hydrophilic carbohydrate probes by the house sparrow *Passer domesticus*. *J. Exp. Biol.* **204**, 723-731.

Chediack, J., Caviedes-Vidal, E., Fasulo, V., Yamin, L. and Karasov, W. (2003). Intestinal passive absorption of water-soluble compounds by sparrows: effect of molecular size and luminal nutrients. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **173**, 187-197.

Crane, R., Miller, D. and Bihler, I. (1961). The restrictions on possible mechanisms of intestinal transport of sugars. In *Membrane Transport and Metabolism* (ed. A. Klenzeller and A. Kotyk), pp. 439-449. New York: Academic Press.

Da Silva, L. C., Da Silva, T. L., Antunes, A. H. and Rezende, K. R. (2015). A sensitive medium-throughput method to predict intestinal absorption in humans using rat intestinal tissue segments. *J. Pharm. Sci.* **104**, 2807-2812.

Diamond, J. M. and Wright, E. M. (1969). Biological membranes: the physical basis of ion and nonelectrolyte selectivity. *Annu. Rev. Physiol.* **31**, 581-646.

Diamond, J. M., Karasov, W. H., Phan, D. and Carpenter, F. L. (1986). Digestive physiology is a determinant of foraging bout frequency in hummingbirds. *Nature* **320**, 62-63.

Dittmann, I., Amasheh, M., Krug, S. M., Markov, A. G., Fromm, M. and Amasheh, S. (2014). Laurate permeates the paracellular pathway for small molecules in the intestinal epithelial cell model HT-29/B6 via opening the tight junctions by reversible relocation of claudin-5. *Pharm. Res.* **31**, 2539-2548.

Ferraris, R. P. and Diamond, J. M. (1986). Use of phlorizin binding to demonstrate induction of intestinal glucose transporters. *J. Membr. Biol.* **94**, 77-82.

Ferraris, R. P. and Diamond, J. (1997). Regulation of intestinal sugar transport. *Physiol. Rev.* **77**, 257-302.

Fiandra, L., Casartelli, M., Cermenati, G., Burlini, N. and Giordana, B. (2009). The intestinal barrier in lepidopteran larvae: Permeability of the peritrophic membrane and of the midgut epithelium to two biologically active peptides. *J. Insect Physiol.* **55**, 10-18.

Fine, K. D., Santa Ana, C. A., Porter, J. L. and Fordtran, J. S. (1993). Effect of D-glucose on intestinal permeability and its passive absorption in human small intestine in vivo. *Gastroenterology* **105**, 1117-1125.

Fine, K. D., Santa Ana, C. A., Porter, J. L. and Fordtran, J. S. (1994). Mechanism by which glucose stimulates the passive absorption of small solutes by the human jejunum in vivo. *Gastroenterology* **107**, 389-395.

Fine, K. D., Santa Ana, C. A., Porter, J. L. and Fordtran, J. S. (1995). Effect of changing intestinal flow rate on a measurement of intestinal permeability. *Gastroenterology* **108**, 983-989.

Gorboulev, V., Schurmann, A., Vallon, V., Kipp, H., Jaschke, A., Klessen, D., Friedrich, A., Scherneck, S., Rieg, T. and Cunnard, R. et al. (2012). Na^+-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. *Diabetes* **61**, 187-196.

Gunzel, D. and Yu, A. S. L. (2013). Claudins and the modulation of tight junction permeability. *Physiol. Rev.* **93**, 525-569.

He, Y.-L., Murby, S., Warhurst, G., Gifford, L., Walker, D., Ayrton, J., Eastmond, R. and Rowland, M. (1998). Species differences in size discrimination in the paracellular pathway reflected by oral bioavailability of poly(ethylene glycol) and D-peptides. *J. Pharm. Sci.* **87**, 626-633.

Hediger, M. A., Coady, M. J., Ikeda, T. S. and Wright, E. M. (1987). Expression cloning and cDNA sequencing of the Na+/glucose co-transporter. *Nature* **330**, 379-381.

Hediger, M.A., Romero, M. F., Peng, J. B., Rolfs, A., Takanaga, H. and Bruford, E. A. (2004). The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins - Introduction. *Pflugers Arch* **447**, 465-468.

Hurni, M. A., Noach, A. R. J., Blom-Roosemalen, M. C. M., De Boer, A., Nagelkerke, J. F. and Breimer, D. D. (1993). Permeability enhancement in Caco-2 cell monolayers by sodium salicylate and sodium taurodihydrofusidate: assessment of effect-reversibility and imaging of transepithelial transport routes by confocal laser scanning microscopy. *J. Pharmacol. Exp. Tech.* **267**, 942-950.

Karasov, W. H. (2011). Digestive physiology: a view from molecules to ecosystem. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **301**, R276-R284.

Karasov, W. H. and Cork, S. J. (1994). Glucose absorption by a nectarivorous bird: the passive pathway is paramount. *Am. J. Physiol.* **267**, G18-G26.

Journal of Experimental Biology

Kellett, G. L. and Helliwell, P. A. (2000). The diffusive component of intestinal glucose absorption is mediated by the glucose-induced recruitment of GLUT2 to the brush-border membrane. *Biochem. J.* **350**, 155-162.
Kellett, G.L., Brot-Laroche, E., Mace, O. J. and Leturque, A. (2008). Sugar absorption in the intestine: the role of GLUT2. *Annu. Rev. Nutr.* **28**, 35-54.
Krause, G., Winkler, L., Mueller, S. L., Haseloff, R. F., Piontek, J. and Blasig, I. E. (2008). Structure and function of claudins. *Biochim. Biophys. Acta* **1778**, 631-645.
Krogh, A. (1929). Progress in physiology. *Am. J. Physiol.* **90**, 243-251.
Lavin, S. R., McWhorter, T. J. and Karasov, W. H. (2007). Mechanistic bases for differences in passive absorption. *J. Exp. Biol.* **210**, 2754-2764.
Lingaraju, A., Long, T. M., Wang, Y., Austin, J. R. I. and Turner, J. R. (2015). Conceptual barriers to understanding physical barriers. *Semin. Cell Dev. Biol.* **42**, 13-21.
Ma, T. Y., Hollander, D., Riga, R. and Bhalla, D. (1993). Autoradiographic determination of permeation pathway of permeability probes across intestinal and tracheal epithelia. *J. Lab. Clin. Med.* **122**, 590-600.
Madara, J. L. and Pappenheimer, J. R. (1987). Structural basis for physiological regulation of paracellular pathways in intestinal epithelia. *J. Membr. Biol.* **100**, 149-164.
McCance, R. A. and Madders, K. (1930). The comparative rates of absorption of sugars from the human intestine. *Biochem. J.* **24**, 795-804.
McWhorter, T. J. and Karasov, W. H. (2007). Paracellular nutrient absorption in a gum-feeding new world primate, the common marmoset *Callithrix jacchus*. *Am. J. Primatol.* **69**, 1399-1411.
McWhorter, T. J., Bakken, B. H., Karasov, W. H. and Martinez del Rio, C. (2006). Hummingbirds rely on both paracellular and carrier-mediated intestinal glucose absorption to fuel high metabolism. *Biol. Lett.* **2**, 131-134.
McWhorter, T. J., Green, A. K. and Karasov, W. H. (2010). Assessment of radiolabeled D-glucose and the nonmetabolizable analog 3-O-methyl-D-glucose as tools for in vivo absorption studies. *Physiol. Biochem. Zool.* **83**, 376-384.
Murer, H., Hopfer, U., Kinne-Saffran, E. and Kinne, R. (1974). Glucose transport in isolated brush-border and lateral-basal plasma-membrane vesicles from intestinal epithelial cells. *Biochim. Biophys. Acta* **345**, 170-179.
Mykles, D. L., Ghalambor, C. K., Stillman, J. H. and Tomanek, L. (2010). Grand challenges in comparative physiology: Integration across disciplines and across levels of biological organization. *Integr. Comp. Biol.* **50**, 6-16.
Naftalin, R. J. (2014). Does apical membrane GLUT2 have a role in intestinal glucose uptake. *F1000Res* **3**, 304.
Noble, D. (2006). *The Music of Life*. New York: Oxford University Press.
Pappenheimer, J. R. (1987). Physiological regulation of transepithelial impedance in the intestinal mucosa of rats and hamsters. *J. Membr. Biol.* **100**, 137-148.
Pappenheimer, J. R. (1990). Paracellular intestinal absorption of glucose, creatinine, and mannitol in normal animals: Relation to body size. *Am. J. Physiol.* **259**, G290-G299.
Pappenheimer, J. R. (1993). On the coupling of membrane digestion with intestinal absorption of sugars and amino acids. *Am. J. Physiol.* **265**, G409-G417.
Pappenheimer, J. R. and Reiss, K. Z. (1987). Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. *J. Membr. Biol.* **100**, 123-136.
Price, E. R., Rott, K. H., Caviedes-Vidal, E. and Karasov, W. H. (2014). Paracellular nutrient absorption is higher in bats than rodents: integrating from intact animals to the molecular level. *J. Exp. Biol.* **217**, 3483-3492.
Price, E. R., Brun, A., Caviedes-Vidal, E. and Karasov, W. H. (2015). Digestive adaptations of aerial lifestyles. *Physiology* **30**, 69-78.
Roder, P.V., Geillinger, K. E., Zietek, T. S., Thorens, B., Koepsell, H. and Daniel, H. (2014). The role of SGLT1 and GLUT2 in intestinal glucose transport and sensing. *PLoS ONE* **9**, e89977.
Rodriguez-Peña, N., Price, E. R., Caviedes-Vidal, E., Flores-Ortiz, C. M. and Karasov, W. H. (2016). Intestinal paracellular absorption is necessary to support the sugar oxidation cascade in nectarivorous bats. *J. Exp. Biol.* **219**, 779-782.
Schultz, S. G. and Curran, P. F. (1970). Coupled transport of sodium and organic solutes. *Physiol. Rev.* **50**, 637-718.
Smulders, A. P. and Wright, E. M. (1971). The magnitude of nonelectrolyte selectivity in the gallbladder epithelium. *J. Membr. Biol.* **5**, 297-318.
Suarez, R. K., Herrera, M. L. G. and Welch, K. C., Jr. (2011). The sugar oxidation cascade: aerial refueling in hummingbirds and nectar bats. *J. Exp. Biol.* **214**, 172-178.
Toloza, E. M. and Diamond, J. (1992). Ontogenetic development of nutrient transporters in rat intestine. *Am. J. Physiol.* **263**, G593-G604.
Tracy, C. R., McWhorter, T. J., Korine, C., Wojciechowski, M. S., Pinshow, B. and Karasov, W. H. (2007). Absorption of sugars in the Egyptian fruit bat (*Rousettus aegyptiacus*): a paradox explained. *J. Exp. Biol.* **210**, 1726-1734.
Uhing, M. R. (1998). Effect of sodium ion coupled nutrient transport on intestinal permeability in chronically catheterized rats. *Gut* **43**, 22-28.
Uhing, M. R. and Kimura, R. E. (1995a). The effect of surgical bowel manipulation and anesthesia on intestinal glucose absorption in rats. *J. Clin. Investig.* **95**, 2790-2798.
Uhing, M. R. and Kimura, R. E. (1995b). Active transport of 3-O-methyl-glucose by the small intestine in chronically catheterized rats. *J. Clin. Investig.* **95**, 2799-2805.
van Wijck, K., Verlinden, T. J. M., van Eijk, H. M. H., Dekker, J., Buurman, W. A., Dejong, C. H. C. and Lenaerts, K. (2013). Novel multi-sugar assay for site-specific gastrointestinal permeability analysis: a randomized controlled crossover trial. *Clin. Nutr.* **32**, 245-251.
Wertheimer, E. (1934). Phloridzinwirkung auf die zuckerresorption. *Pfluger's Arch. Gesamte Physiol. Menschen Tiere* **233**, 514-528.
Wright, E. M., Van Os, C. H. and Mircheff, A. K. (1980). Sugar uptake by intestinal basolateral membrane vesicles. *Biochim. Biophys. Acta* **597**, 112-124.
Wright, E. M., Martin, M. G. and Turk, E. (2003). Intestinal absorption in health and disease—sugars. *Best Practice Res. Clin. Gastroenterol.* **17**, 943-956.
Wright, E. M., Loo, D. F. and Hirayama, B. A. (2011). Biology of human sodium glucose transporters. *Physiol. Rev.* **91**, 733-794.
Yuasa, H., Matsuda, K., Kimura, Y., Soga, N. and Watanabe, J. (1997). Comparative assessment of intestinal transport of hydrophilic drugs between small intestine and large intestine. *Drug Deliv.* **4**, 269-272.
Zhu, Q., Liu, Z., Li, P. and Cheng, Z. (2014). Drug interaction studies reveal that Simotinib upregulates intestinal absorption by increasing the paracellular permeability of intestinal epithelial cells. *Drug Metab. Pharmacokinet* **29**, 317-324.