Allometry of Paracellular Absorption in Birds

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ABSTRACT

Water-soluble nutrients can be absorbed across the intestinal epithelium by transcellular and paracellular processes. Recent studies suggest that small birds (<180 g) have more extensive paracellular absorption of glucose than nonflying mammals. This may be a feature that compensates for a reduced small intestine size because small birds have smaller mass-corrected intestinal length than do nonflying mammals, but the difference diminishes in larger birds. We hypothesized that if this explanation were correct, there would be a negative correlation between paracellular absorption and body mass in birds and that larger birds would have paracellular absorption comparable to that of nonflying mammals. We tested this hypothesis, using consistent methodology, by measuring the extent of absorption of a series of inert carbohydrate probes in heavier bird species (>300 g) selected from diverse taxa: American coots, mallards, pheasants, and pigeons. Absorption of carbohydrate probes was inversely related to body mass in birds, and absorption of these probes in large birds (>500 g) was comparable to absorption measurements in nonflying mammals. Higher paracellular uptake in the smaller avian species may offer a physiologically inexpensive means of nutrient absorption to compensate for a reduced small intestine size but may make those species more vulnerable to toxicant absorption.

Introduction

Water-soluble nutrients (e.g., glucose) can be absorbed across the intestinal epithelium by transcellular and paracellular processes. Transcellular absorption of compounds such as carbohydrates and amino acids occurs with the aid of specific membrane-bound proteins that transport these compounds across the apical and basolateral membranes of intestinal cells. In contrast, paracellular solute transport occurs through junctions between adjacent enterocytes rather than across their membranes, is passive, and does not exhibit saturation kinetics. Thus, paracellular nutrient absorption rate is matched to dietary load, and efficiency of absorption (i.e., fractional absorption) is independent of dietary load (Pappenheimer 1993; Karasov and Cork 1994).

Paracellular uptake of water-soluble chemicals occurs by diffusion or by way of solvent drag, and in the latter case, transcellular absorption of osmotically active compounds stimulates water and dissolved-solute movement across “tight” junctions (Pappenheimer and Reiss 1987; Pappenheimer 1993). These tight junctions are composed of interlaced protein strands that function as a sieve (Tsukita and Furuse 2000; Anderson 2001). The paracellular route of absorption of water-soluble compounds has been visualized by either autoradiography (of polyethylene glycol; Ma et al. 1993) or confocal laser microscopy (Hurni et al. 1993; Sakai et al. 1997; Chang and Karasov 2004a). Its molecular size selectivity has been characterized by measuring absorption of a series of nonelectrolyte water-soluble probes that differ in molecular dimension (Hamilton et al. 1987; Ghandehari et al. 1997; Chediack et al. 2003), and its charge selectivity has been characterized through the use of relatively inert charged peptides (He et al. 1998; Chediack et al. 2006). While paracellular absorption offers a relatively inexpensive means of nutrient uptake because it matches dietary load without necessitating upregulation of nutrient transporters, it is not selective except in regard to size and charge; consequently, animals that have extensive paracellular absorption may be exposed to greater systemic concentrations of water-soluble toxins found in the diet (Diamond 1991).

All measures of high paracellular nutrient absorption have been in small birds (<200 g; McWhorter 2005). For example, fractional absorption of l-glucose (the stereoisomer of D-glucose not absorbed transcellularly; Chang et al. 2004) was extensive in several small avian species: 79% in house sparrows (Passer domesticus, Passeridae, mean body mass 26 g; Chang and Karasov 2004b); 65% in broad-tailed hummingbirds (Cyananthus latirostris, Trochilidae, 2.5 g; McWhorter et al. 2006); 80% in rainbow lorikeets (Trichoglossus haematodus, Psittacidae, 122 g; Karasov and Cork 1994); 91% in yellow-rumped warblers (Dendroica coronata, Parulidae, 12 g; Afik et al. 1997); and 92% in northern bobwhites (Colinus virginianus, Odontophoridae, 167 g; Levey and Gipollini 1996). Furthermore, house sparrows had similar fractional absorption (80%) and apparent rates of absorption for l-glucose and 3-O-methyl-D-glucose (a nonmetabolizable analog of D-glucose that is absorbed both paracellularly and transcellularly via the D-glucose transporter), suggesting that the majority of glucose absorption...
in this species is passive (Chang and Karasov 2004b). The large proportion of D-glucose that was absorbed in these small species of birds from five different avian families with varied diets (omnivores, nectarivores, and granivores) suggests that paracellular absorption is generally an important pathway of transport of water-soluble compounds in small avian species.

While small bird species have extensive paracellular transport, nonflying mammals of varying body masses studied to date do not. Paracellular absorption contributes little (<7%) to glucose absorption in rats (Schwartz et al. 1995; Uling and Kimura 1995), dogs (Lane et al. 1999), humans (Fine et al. 1993), and rhesus macaques (Macaca mulatta) and common marmosets (Callithrix jacchus; Karasov et al. 2006; McWhorter and Karasov 2006). Results from urinary recovery and intestinal perfusion experiments measuring inert carbohydrate absorption in these mammalian species suggest that the majority (>93%) of glucose transport is via a carrier-mediated process across the brush border membrane of the intestinal epithelium and that only a small fraction is absorbed across the tight junction.

Could differences in routes of absorption between birds and mammals be a consequence of flight? A survey of the literature indicated that birds have significantly less small intestine nominal surface area (area of a smooth-bore tube) and that small birds (<365 g) have significantly shorter small intestines than comparably sized nonflying mammals (Karasov and Hume 1997; Lavin 2007; Lavin et al. 2008). A smaller gut and, consequently, a smaller digesta volume reduce the cost of flight, and takeoff and maneuverability may be diminished or impaired at heavier masses (Guillemette 1994; Norberg 1995; Nudds and Bryant 2002); thus, nominal surface area, a function of circumference that also corresponds to gut volume, may be reduced in volant species because of selection pressure. Although there is evidence that intestinal villi increase surface area to a slightly (by ca. 15%) greater extent in birds than in mammals, that does not entirely compensate for the much greater reduction in nominal surface area (Lavin 2007; Lavin et al. 2008).

In addition, there is no evidence for a compensatory increase in the number of transporters in the brush border of the intestine or the rate of mediated transport of D-glucose or amino acids per unit nominal or villus area in birds compared with nonflying mammals (Karasov and Hume 1997; Lavin 2007; Lavin et al. 2007). For example, in experiments using everted sleeves of intestine, mediated uptake rates of D-glucose in the rat were about double that of the pigeon whether normalized to mass, length, or nominal surface area (Lavin et al. 2007). Furthermore, there was no significant difference between omnivorous birds and mammals in transporter-mediated uptake rate of D-glucose or total amino acid l-proline uptake per unit nominal surface area of small intestine (n = 7 and 8 bird and mammal species, respectively; Karasov and Hume 1997). Measurements of nutrient uptake standardized per unit nominal intestine area inherently take differences in surface amplification into account and do not differ significantly between birds and mammals, suggesting that total capacity for mediated uptake is lower in birds. Thus, the difference in total intestinal surface area between birds and nonflying mammals is not likely to be counterbalanced by greater digestive surface amplification by villi in birds, at least in the case of mediated nutrient uptake.

Why smaller birds might have more selection pressure to minimize gut volume is not clear; but whatever the reason(s), paracellular absorption may be a compensatory mechanism allowing efficient nutrient assimilation for these small bird species. Because small intestine length in large birds (>365 g) is not significantly different from that in mammals (Lavin 2007), perhaps the difference in paracellular absorption between birds and mammals is not so large at large body masses. No study has measured paracellular absorption in bird species larger than 200 g. We hypothesized that the extent of paracellular absorption in larger avian species would be less than that in smaller birds and more comparable to that in nonflying mammals. Specifically, from the allometry of small intestine length, we predict that birds larger than 300–400 g will have significantly less paracellular absorption than smaller birds studied to date, that is, a fraction not significantly different from that in nonflying mammals.

In order to test this hypothesis, we measured the fractional absorption of a series of inert (not metabolized and not actively transported) carbohydrate probes of three sizes (l-arabinose, MW [molecular weight] = 150 Da; l-rhamnose, MW = 164 Da; and cellobiose, MW = 342 Da) in three large (>500 g), omnivorous bird species from different phylogenetic superorders: pheasants (Phasianus colchicum; Galliformes), mallards (Anas platyrhynchos; Anseriformes), and American coots (Fulica americana; Gruiformes). We chose species in relatively distantly related taxonomic groups in order to increase the generality of our finding. We also quantified absorption of l-arabinose and l-rhamnose in a midsized (300-g) bird, the pigeon (Columba livia; Columbiformes) and compared measures of paracellular absorption in bird species from this study and the literature with published mammalian records. The carbohydrate probes we used in our study are commonly used in tests of passive (non–carrier-mediated) intestinal permeability (in humans; reviewed by Travis and Menzies [1992]) and are not absorbed transcellularly in pigeons (Lavin et al. 2007), and they differ in molecular size, which permits some determination of paracellular absorption’s molecular-size selectivity. In order to achieve the molecular-size range, we used both monosaccharides and disaccharides as inert probes.

Material and Methods

Animals and Their Maintenance

Six pigeons (Columba livia, mean body mass ± SEM = 270 ± 8 g), 10 pheasants (Phasianus colchicus; mean body mass ± SEM = 938 ± 65 g), and eight mallards (Anas platyrhynchos; mean body mass ± SEM = 1,237 ± 36 g) were purchased from Wisconsin vendors/breeders (pigeons: Earl Ditsch Farm; pheasants: Hillside Springs Game Farm; mallards:
Experimental Procedures

Carbohydrate probes were purchased from Sigma Chemicals (St. Louis, MO): l-arabinose (C5H7O5; MW = 150.13 Da), l-rhamnose (C6H12O5; MW = 156.2 Da), and cellobiose (C12H22O11; MW = 342.3 Da). Pheasants, mallards, and coots were gavaged (force-fed) with a solution 363 ± 3 mmol/kg (measured using a Wescor 5100 vapor pressure osmometer), isosmotic to avian plasma (Goldstein and Zahedi 1990), containing 25 mM NaCl, 50 mM 3-O-methyl-d-glucose, 50 mM l-arabinose, 50 mM l-rhamnose, and 150 mM cellobiose at a dose of 1.4% body mass. These birds were injected in the pectoralis muscle with the same solution (0.6% body mass) in order to expel the hydrogen gas evolved during the derivatization reaction. The carbohydrates in derivitized plasma samples were separated by HPLC (Beckman-Coulter 508 autosampler, System Gold 126 solvent module, 32 Karat software, ver. 5.0, Build 1021; Beckman-Coulter, Fullerton, CA). Twenty microliters of derivitized plasma samples was injected on a C-18 reversed-phase column (Waters Pico Tag, ; Waters, Milford, 150 mm # 1 anthranilic acid and 20 mg mL−1 sodium cyanoborohydride dissolved in a previously prepared solution of 5% sodium acetate · 3H2O and 2% boric acid in methanol. Samples were transferred to a screw-cap glass autosampler vial and heated at 65°C for 3 h. After samples were cooled to ambient temperature, 300 μL of HPLC solvent A (see below) was added to vials; vial contents were mixed vigorously in order to expel the hydrogen gas evolved during the derivatization reaction.

Blood samples were centrifuged, and the resulting plasma samples were loaded into preweighed 1.5-mL microcentrifuge tubes equipped with 30K Nanosep filters (Pall, East Hills, NY). Plasma was initially filtered with 50 μL of distilled water (dH2O; 14,000 g for 30 min), and then rinsed with an additional 100 μL of dH2O (14,000 g for 140 min) to ensure high carbohydrate probe recovery. Samples were subsequently dried at 65°C and stored frozen until analysis.

Carbohydrate probes in plasma samples were derivatized for high-performance liquid chromatography (HPLC) fluorescence detection by reductive amination with anthranilic acid (2-amino-5-nobenzoic acid), following Anumula (1994) and Du and Anumula (1998) with minor modifications. Dried plasma samples were reconstituted with 50 μL of dH2O and mixed with 50 μL of anthranilic acid reagent solution. The anthranilic acid reagent consisted of 30 mg mL−1 anthranilic acid and 20 mg mL−1 sodium cyanoborohydride dissolved in a previously prepared solution of 5% sodium acetate · 3H2O and 2% boric acid in methanol. Samples were transferred to a screw-cap glass autosampler vial and heated at 65°C for 3 h. After samples were cooled to ambient temperature, 300 μL of HPLC solvent A (see below) was added to vials; vial contents were mixed vigorously in order to expel the hydrogen gas evolved during the derivatization reaction.

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ratio of each solvent linearly over 23 min. Carbohydrate probes in plasma samples were quantified by a fluorescence spectrophotometer with the following settings: excitation wavelength 230 nm, slit width 10 nm; emission wavelength 425 nm, slit width 5 nm; sensitivity = 1; “normal” settings for lamp mode, photomultiplier gain, and response time (Anumula 1994; PerkinElmer 650-LC; PerkinElmer Life Sciences, Boston). Plasma carbohydrate concentration was calculated using a standard curve \( r > 0.99 \) generated by measuring each derivitized carbohydrate along a concentration gradient bracketing both high and low physiological concentrations. Each standard curve automatically corrected for the efficiency in the derivatizations and the sample processing dilution factor.

Pharmacokinetic Calculations of Absorption

The plasma concentrations \( C \) (ng probe mg\(^{-1}\) plasma) were plotted as a function of sample time \( t \) (minutes). The amounts of paracellular probes absorbed were calculated from the areas under the postgavage and postinjection plasma curves (AUC, area under the curve of plasma probe concentration vs. time). Fractional absorption \( f \) was calculated as \( \frac{\text{AUC by gavage/dose}_{\text{gavage}}}{\text{AUC by injection/dose}_{\text{injection}}} \) (Ritschel 2004). This method of calculating \( f \) relies on no major assumptions about compartments or kinetics, and it accounts for \( <100\% \) recovery of probes, should that occur, or for differences in distribution space, assuming that gavaged and injected amounts behave similarly with regard to postadministration distribution and recovery. The AUC from \( t = 0 \) to \( t = x \) min (time of final blood sampling) was calculated using typical pharmacokinetic procedures (Ritschel 2004) and the trapezoidal rule. The AUC from \( t = x \) min to \( t = \infty \) was calculated by dividing \( C_t \) (plasma concentration at time of final blood sampling) by \( K_{el} \) which is the rate constant describing the probe loss from the systemic circulation by elimination. This parameter was estimated by regressing the last several log-transformed plasma concentrations \( C \) against \( t \) and calculating the slope.

Statistical Analysis

Results are expressed as means ± SEM. Statistical analyses were conducted in Systat (ver. 10; Systat Software, Point Richmond, CA). Estimates of \( f \) were arcsine–square root transformed before statistical analyses. Repeated-measures ANOVA was used to test for differences in elimination rate constants and in probe absorption within and among species. An ANCOVA was used to determine whether fractional absorption was body mass dependent (this analysis included data from Chediack et al. 2003).

In an even broader comparative analysis, we included data from the literature on fractional absorption of many neutral paracellular probes in birds and nonflying mammals (see the appendix in the online edition). In the comparison of taxa, we did not distinguish between measurements of fractional absorption that were made in the presence of luminal nutrients and those made in their absence (i.e., in fasted animals). Although permeability of the paracellular pathway is increased when \( \text{Na}^+ \)-coupled glucose and amino acid transport occurs (Pappenheimer and Reiss 1987; Sadowski and Meddings 1993; Turner and Madara 1995), the increase in fractional absorption in rats and house sparrows \( (0.06 ± 0.03, n = 4 \text{ studies with } \text{l-rhamnose, } \text{l-glucose, and mannitol } \text{(Chediack et al. 2003; Chang et al. 2004; Lavin et al. 2004)}) \) was small relative to the difference in fractional absorption between birds and nonflying mammals. In order to test for the effect of probe size, we used a linear regression of log-transformed molecular weight and arcsine–square root–transformed fractional absorption. We then conducted an ANCOVA on the residuals of this regression to test for significant effects of taxa (bird vs. nonflying mammal), log-transformed body mass, and the interaction between taxa and body mass.

Results

After injection and gavage, probes were cleared rapidly, with \( >90\% \) of elimination occurring over the course of the 1.5-h experiment (4 h in pigeons and mallards; Fig. 1). Fitting the elimination data to a monoeponential elimination model gave values of \( r^2 > 0.98 \) for \( \text{l-rhamnose, } \text{l-arabinose, and cellobiose} \) that support our method of estimating the residual AUC past 90 min (in pheasants and coots) or 240 min (in pigeons and mallards) using the apparent elimination rate constants \( (K_{el}; \text{see “Pharmacokinetic Calculations of Absorption”}) \). The birds’ postgavage and postinjection \( K_{el} ' s \) were not significantly different for any of the probes (Table 1). There was no significant difference in fractional absorption of \( \text{l-arabinose, } \text{l-rhamnose, or cellobiose} \) among mallards, pheasants, and coots, but pigeons had significantly greater absorption of \( \text{l-arabinose and l-rhamnose} \) than mallards, pheasants, and coots. Birds also absorbed smaller probes significantly more than larger probes (Table 1). For just \( \text{l-arabinose} \) and \( \text{l-rhamnose} \) (these measurements were performed in all birds in this study), \( \text{l-arabinose} \) was absorbed to a greater extent than \( \text{l-rhamnose} \) \( (F_{48} = 16.33, P < 0.001) \), and probe size selectivity occurred for all bird species (e.g., no significant interaction term; probe × species: \( F_{3,48} = 1.70, P = 0.18) \).

There was a significant effect of body mass \( (F_{1.6} = 20.59, P = 0.004) \) on fractional absorption (arcsine–square root–transformed ANCOVA) of \( \text{l-arabinose and l-rhamnose} \); smaller bird species (sparrows and pigeons) had greater absorption of these small paracellular probes than larger species (mallards, pheasants, and coots). The effect of body mass occurred for both \( \text{l-arabinose} \) and \( \text{l-rhamnose} \) (e.g., no significant interaction term; probe × body mass: \( F_{1.6} = 0.07, P = 0.80) \). A linear regression showed that fractional absorption of \( \text{l-arabinose} \) and \( \text{l-rhamnose} \) was significantly dependent on body mass (pooled slope = \( -0.13), \) lower 95\% confidence interval \( [CI] = -0.20, \) upper 95\% CI \( = -0.07, \) \( F_{1.7} = 0.002); however, fractional absorption of cellobiose or lactulose was uniformly low and independent of body mass; \( F_{1.7} = 1.01, P = 0.42) \).
Figure 1. l-arabinose (a), l-rhamnose (b), and cellobiose (c) injected (solid lines) or gavaged (dashed lines) into pigeons, coots, pheasants, and mallards were cleared from the blood rapidly. Insets show probe concentrations for the final three or four blood-sampling times (t = 90, 150, and 240 min for pigeons and mallards; t = 30, 45, 60, and 90 min for coots; t = 45, 60, and 90 min for pheasants), with lines representing least square fits on a semilogarithmic plot. The data points represent means ± SEM (n = 6 pigeons, 8 coots, 8 mallards, and 8 pheasants).

When we included values from the literature on nonflying mammals and additional avian species in the analysis, smaller probes were again absorbed more readily than larger probes ($F_{1,56} = 26.31$, $P < 0.001$; Fig. 3A). There was also a significant effect of taxa (bird vs. nonflying mammal; $F_{1,56} = 32.93$, $P < 0.001$), body mass ($F_{1,56} = 21.14$, $P < 0.001$), and the interaction between taxa and body mass ($F_{1,56} = 17.97$, $P < 0.001$) on the residuals of fractional absorption of paracellular probes (Fig. 3B).

Discussion

As we hypothesized, birds larger than 300–400 g had little paracellular absorption of small water-soluble probes. Paracellular
<table>
<thead>
<tr>
<th>Species, Probe</th>
<th>Elimination Rate Constant ($K_{el}$; mean ± SEM)</th>
<th>Effect on $K_{el}$</th>
<th>Effect of Probe on $f$</th>
<th>Effect of Species on $f$</th>
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<tbody>
<tr>
<td><strong>Pheasant, n = 8:</strong></td>
<td></td>
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<tr>
<td>L-arabinose</td>
<td>$-0.19 ± 0.02$</td>
<td>$-0.11 ± 0.02$</td>
<td>$0.31 ± 0.05$</td>
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<tr>
<td>L-rhamnose</td>
<td>$-0.16 ± 0.02$</td>
<td>$-0.11 ± 0.03$</td>
<td>$0.15 ± 0.03$</td>
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<tr>
<td>Cellobiose</td>
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<td>$-0.20 ± 0.008$</td>
<td>$0.19 ± 0.03$</td>
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<td><strong>Mallard, n = 8:</strong></td>
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<tr>
<td>L-arabinose</td>
<td>$-0.14 ± 0.04$</td>
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<td>L-rhamnose</td>
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<td>Cellobiose</td>
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<td><strong>Coot, n = 8:</strong></td>
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<td>L-arabinose</td>
<td>$-0.36 ± 0.010$</td>
<td>$-0.23 ± 0.003$</td>
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<td>L-rhamnose</td>
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<td>Cellobiose</td>
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<td><strong>Pigeon, n = 6:</strong></td>
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<td>L-arabinose</td>
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<td>L-rhamnose</td>
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<td>$0.38 ± 0.04$</td>
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* Repeated-measures ANOVA for differences in $K_{el}$ measured with different probes postinjection and postgavage.

* Repeated-measures ANOVA and paired t-test (pigeons) for differences in $f$. 
Figure 2. Fractional absorption of l-arabinose (molecular weight $[\text{MW}] = 150.1$ Da; circles), l-rhamnose (MW = 164.2 Da; crosses), and cellobiose (MW = 342.3 Da; both cellobiose and lactulose are represented by plus signs) in sparrows (data from Chediack et al. 2003), pigeons, coots, pheasants, and mallards. Each point represents the fractional absorption of a probe by a species. Fractional absorption of l-arabinose and l-rhamnose was significantly dependent on body mass ($\text{slope} = -0.13$, lower 95% confidence interval [CI] = $-0.20$, upper 95% CI = $-0.07$).

Figure 3. Fractional absorption of neutral inert water-soluble compounds from this study and in the literature (see the appendix in the online edition for citations) for birds (circles; 22 measurements on 10 species) and nonflying mammals (crosses; 36 measurements on 11 species). In A, the fractional absorption (arcsine-square root transformed) is regressed against probe molecular weight. In B, the residuals from that regression are plotted as a function of log-transformed body mass. See the last paragraph of “Results” for statistical details.

absorption of the smaller probes l-arabinose and l-rhamnose decreased linearly with bird body mass, but absorption of the larger probes cellobiose and lactulose was independent of body mass. Furthermore, considerable (>50%) absorption of l-glucose has been found in several small bird species (<175 g), including house sparrows (Chang and Karasov 2004b), broad-tailed hummingbirds (McWhorter et al. 2006), rainbow lorikeets (Karasov and Cork 1994), yellow-rumped warblers (Afik et al. 1997b), and northern bobwhites (Levey and Cipollini 1996).

When we included data from the literature on fractional absorption of many neutral paracellular probes in birds and nonflying mammals, we found that small birds have more extensive paracellular absorption than large birds and all nonflying mammals and that the differential between birds and nonflying mammals in fractional absorption of paracellular probes declines with increasing body size. These trends were also evident when a subset of the data collected using methodology comparable to that of our study was used in comparable analyses (data not shown). Thus, our predicted taxonomic trends were robust despite differences in experimental methodology among studies.

The paracellular pathway of nutrient absorption may be a compensatory mechanism for small bird species to assimilate energy. Minimizing the mass associated with carrying a large gut load may be advantageous for minimizing the metabolic costs associated with flight. For example, the hourly rate of metabolism of racing pigeons wearing a load 2.5% or 5.0% of their body weight was 41%–52% higher during a flight than that of pigeons not wearing an additional load; however, the authors caution that these are likely overestimates for avian species in the wild (Gessaman and Nagy 1988). Furthermore, diminished or impaired takeoff and maneuverability at heavier masses may increase predation risk; survivorship of male rock ptarmigans (Lagopus mutus) fitted with radio transmitters weighing 3.6% of the birds’ body mass was lower than that of unmarked males (Cotter and Gratto 1995). A smaller digesta weight burden, and thus a smaller intestine volume, however, would reduce the surface area for the active transport of nutrients. In yellow-rumped warblers, rainbow lorikeets, house
sparrows, and northern bobwhites (but not in mice, rats, or rabbits; Ferraris and Diamond 1989), the capacity of mediated transport of \( \text{d}-\text{glucose} \) measured in vitro underestimated the total glucose uptake at the whole-animal level, suggesting the use of an alternative absorptive pathway, and correspondingly, paracellular absorption measured in intact animals was high (Karasov and Cork 1994; Caviedes-Vidal and Karasov 1996; Karasov et al. 1996; Levey and Cipollini 1996; Afik et al. 1997a, 1997b; Chang and Karasov 2004).

Our data and those from the literature suggest that paracellular absorption is negatively correlated to body mass in birds, although we cannot specify whether the paracellular absorption occurs mainly by diffusion or by solvent drag (paracellular solute absorption concomitant with water absorption). Our analysis contrasts with that of Pappenheimer (1998), who concluded that the ratio of solvent drag to carrier-mediated transport increased (vs. decreased) with body mass among mammal species. We used a uniform methodology to measure paracellular absorption among omnivorous avian species of varying masses and found no evidence for positive scaling in birds.

The mechanism underlying enhanced passive transport remains largely uncertain (Lavin et al. 2007). Smaller birds, such as the house sparrow (Chediac et al. 2003) and pigeon (this study), had significantly higher passive absorption of smaller water-soluble probes than larger birds, whereas sparrows (Chediac et al. 2003) and the large bird species had comparable low fractional absorption of the probe with the MW of 342 Da. One explanation for this finding might be that the effective pore size of the tight junction between enterocytes may not be markedly different among these avian species, making larger effective pore size a less likely mechanism for enhanced paracellular absorption. The tight junction is composed of a network of assorted protein species that form pores of various permeability, depending on organ tissue; tight-junction leakiness may be a function of the number of strands (protein particles) and the combination and mixing ratio of different proteins in the tight junction (Tsukita and Furuse 2000). Because sparrows and large bird species have comparable low fractional absorption of the larger probe, the effective pore size of their tight junctions may be equivalent, and thus, the molecular weight cutoff for passive permeability in these birds may be the same. Differences in intestinal morphology, such as larger nominal surface area, larger villus area, or a greater density or number of tight junctions, could also offer enhanced passive transport; however, these characteristics were not markedly different among avian species (Lavin 2007).

Extensive paracellular absorption of water-soluble chemicals seems to be a phenomenon of small avian species compared to nonflying mammals and larger species of birds. Greater paracellular absorption in small birds may help balance the effects of a significantly reduced small intestine with which to actively absorb nutrients (Lavin et al. 2008). Although our study was performed on a small number of bird species ( \( n = 5 \) ), our species represent a diverse group taxonomically, and our study controls for diet in that these species are omnivores consuming foodstuffs such as plant material and insects.

The similar low paracellular absorption in larger avian species is not an artifact of sampling within a phylogenetic line; our species are from three different taxonomic superorders (Galliformes for pheasants, Anseriformes for mallards, and Passeriformes for pigeons, coots, and house sparrows). Conversely, the variation in paracellular absorption with body size is apparent within the single superorder Passerimorphae. House sparrows (order Passeriformes) had significant passive absorption, pigeons (order Columbiformes) had less, and coots (order Gruiformes) exhibited little passive absorption. Furthermore, within order Galliformes, we also note that passive absorption declines with increasing body mass. On the basis of \( \text{L}-\text{glucose} \) absorption, more glucose was absorbed passively in northern bobwhite quail (body mass = 180 g; order Galliformes; Levey and Cipollini 1996) than in pheasants (938 g; order Galliformes; this study).

There are both costs and benefits of enhanced small intestine permeability to water-soluble chemicals. Passive absorption offers a physiologically inexpensive means of nutrient absorption; uptake capacity is matched to dietary load (Pappenheimer 1993; Karasov and Cork 1994). But this route of absorption is relatively less selective than mediated transport, and it may make certain taxa or species more vulnerable to absorption of water-soluble toxicants or compounds found in plants (Diamond 1991), such as simple phenolics (gallic acid; Konishi et al. 2003), flavonoids, alkaloids (e.g., caffeine; Leahy et al. 1994), and non-protein amino acids. Perhaps species with high paracellular absorption avoid such chemicals, although they may still be exposed to human-made compounds and/or have evolved enhanced detoxification processes to handle an increased systemic toxicant load.

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Literature Cited


