Intestinal passive absorption of water-soluble compounds by sparrows: effect of molecular size and luminal nutrients

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Abstract We tested predictions that: (1) absorption of water-soluble probes decreases with increasing molecular size, consistent with movement through effective pores in epithelia, and (2) absorption of probes is enhanced when measured in the presence of luminal nutrients, as predicted for paracellular solvent drag. Probes (L-arabinose, L-rhamnose, persitol, lactulose; MW 150.1–542.3 Da) were gavaged in nonanesthetized House sparrows (Passer domesticus), or injected into the pectoralis, and serially measured in plasma. Bioavailability was calculated as $F = \frac{AUC}{AUC_{by}}$ by gavage/AUC by injection, where AUC is the area under the curve of plasma probe concentration vs. time. Consistent with predictions, $F$ declined with probe size by 75% from the smallest to the largest probe, and absorption of probes increased by 40% in the presence of luminal glucose or food compared to a mannitol control. Absorption of water-soluble probes by sparrows is much higher than in humans, which is much higher than in rats. These differences seem mainly attributable to differences in paracellular solvent flux and less to differences in effective paracellular pore size.

Keywords Carbohydrates · Modulation · Molecular size · Paracellular absorption · Small intestine

Abbreviations $a$: molecular radius · $AUC$: area under the curve · $C_t$: concentrations of solute at two ends of the channel · $F$: fractional absorption · $PEG$: polyethylene glycol · $J$: total solute flux · $r$: flow of solvent · $r_p$: pore radius · $S_{m}$: intestinal surface area · $t$: treatment · $C$: control treatment · $t^*$: treatment $F$ food treatment · $t^*$: treatment $G$ glucose treatment

Introduction

Generally, the transport of molecules across the phospholipid bilayer membrane of intestinal enterocytes is correlated with their lipid-water partition coefficient (Diamond and Wright 1969; Smulders and Wright 1971). This membrane is thus absorption limiting for water-soluble molecules. Yet, there are reports in mammals and birds of considerable absorption of small to medium-sized water-soluble compounds such as creatinine (Pappenheimer 1990), carbohydrates (Caviedes-Vidal and Karasov 1996; Chediac et al. 2001; Hamilton et al. 1987; Karasov and Cork 1994), lipid-insoluble octopeptides (He et al. 1996; Pappenheimer et al. 1994), polyethylene glycol (He et al. 1998; Ma et al. 1993), and inulin (Ma et al. 1995). It has been presumed that these water-soluble molecules permeate across the small intestinal mucosal epithelium primarily through the paracellular pathway (Madora and Pappenheimer 1987; Pappenheimer 1987; Pappenheimer and Reiss 1987; Powell 1987). Autoradiography (Ma et al. 1993) and confocal laser scanning microscopy (Hurni et al. 1993) have visualized the appearance of water-soluble probes in the paracellular space. The major physical structure defining the permeability properties of the paracellular barrier is the tight junction (Anderson 2001; Ballard et al. 1995). Thus the flux of solutes through the paracellular pathway should be determined by both the chemical and physical properties of the molecules (e.g., size, electroaffinity) and the tight junction. The barrier is created where protein particles (fibrils or "strands") in plasma membranes of adjacent cells meet in the paracellular
space. Aquous pores are thought to exist within the paired strands (Tsuchita and Furuse 2000), and this is the putative path for water-soluble compounds. As described below, this pathway should discriminate according to molecular size and it can also be modulated; these are the features we studied using house sparrows (Passer domesticus).

Several studies have evaluated the passive permeability characteristics of the small intestine using a series of nonelectrolyte water-soluble probes that differ in molecular dimensions, such as inert carbohydrates (Ghandehari et al. 1997; Hamilton et al. 1987) or polyethylene glycol (PEG) of varying molecular weights (He et al. 1998; Meheye 1996). In these studies, absorption declined with increasing molecular weight of probes more rapidly than the free aqueous diffusion coefficients of the probes, consistent with movement through effective pores in epithelia ("sewing"; Chang et al. 1975; Friedman 1987). However, a cautionary warning about interpreting experimental phenomena in physical terms applies (Friedman 1987; Schultz 1980): the "pores" may be tortuous channels not necessarily characterized by a single radius. Nonetheless, the data give an indication of the size selectivity of the pathway and, functionally, this seems important insofar as it partly determines the size range of water-soluble nutrients or toxins that might be absorbed along this pathway. In our study of house sparrows we selected a series of carbohydrate probes including, for comparison, some of those already studied in humans and rats. We thought that the virtue of the series of carbohydrate probes in comparison with PEG oligomers is that the former mainly increase in molecular radius (imagine larger and larger spheres composed of increasing numbers of carbon atoms) whereas the latter mainly increase in length. Also, there is some controversy about whether PEG oligomers are somewhat lipophilic and might therefore cross the apical membrane to some extent (Bjarnason et al. 1995).

The second major objective of our study was to test for modulation of paracellular absorption of water-soluble compounds. Several studies have documented relatively rapid changes in paracellular permeability, apparently triggered by endogenous agents such as cAMP (Perez et al. 1997), cytokines and leukocytes (Nusrat et al. 2000), and exogenous agents that include dietary constituents such as glucose and amino acids (Madara and Pappenheimer 1987; Pappenheimer 1987; Pappenheimer and Reiss 1987; Pappenheimer and Volpp 1992; Sadowski and Meddings 1995), medium chain fatty acids (Lindmark et al. 1998) and natural toxins such as capsaicinoids, a diterpene glucoside from sweet pepper (Shimizu 1999) and the alkaloid theophylline (Perez et al. 1997). The mechanism(s) is not known, but might be increased solvent drag and/or cytoskeletal contractions (Madara and Pappenheimer 1987; Madara et al. 1986, 1988; Pappenheimer 1987; Pappenheimer and Reiss 1987) or protein strand alterations that alter the tight junction effective pore size. In our study with house sparrows, we provide the first test of modulation of apparent paracellular transport of a series of water-soluble probe molecules, using both luminal food and glucose as test stimulatory agents.

We studied house sparrows because their digestive tract is relatively simple (oesophagus, stomach, intestine) and their food processing is relatively rapid. Therefore, in short fairly non-invasive whole animal experiments we can measure digestive processes that occur primarily in the stomach and small intestine. Furthermore, their cosmopolitan distribution and ease of capture and laboratory maintenance make house sparrows good subjects for a variety of laboratory studies of avian physiology, although we will also compare our results with those from mammal studies. For two reasons our experiments measure absorption by the intact animal rather than by intestine in situ in anesthetized animals or by tissue preparations. First, while the latter methods can demonstrate conditions and mechanisms by which absorption can occur, they cannot by themselves demonstrate the mechanisms by which it normally occurs. Second, there is evidence that approaches not relying on intact animals may introduce artifacts (e.g., Uning and Kimura 1995a, 1995b). Students of intestinal transport have historically relied on a variety of in vitro and in vivo methods to assemble their picture of how the intestine absorbs solutes. Our studies, and others on intact animals (Bijlsma et al. 1995; Delahunty and Holland 1987; He et al. 1990; Pappenheimer 1990; Turner and Madara 1995), complement those on paracellular absorption that rely on perfusion of anesthetized animals (Fagerholm et al. 1999; Hamilton et al. 1987; Leathy et al. 1994; Perez et al. 1993) and measures in either intact tissue (Ghandehari et al. 1997) or cell cultures (Adson et al. 1994; Karlsson et al. 1999; Knipp et al. 1997), and help assess the importance of passive absorption in whole animals under fairly natural conditions.

The two major predictions we tested were that: (1) absorption of water-soluble carbohydrate probes would progressively decrease with increasing molecular size up to the terminal size beyond which larger molecules are essentially excluded; and (2) absorption of all probes would be enhanced when measured in the presence of luminal glucose or food as compared with measures in the absence of nutrients. To measure absorption we used a pharmacokinetic technique that relies on the appearance of probes in blood and which involves feeding and injecting probes and then sampling blood at various times post-gavage or post-injection (Caviedes-Vidal and Karasov 1996; Chedzick et al. 2001).

Materials and methods

Birds and their maintenance

Fourteen house sparrows (P. domesticus) were captured with live traps in the vicinity of the Universidad Nacional de San Luis Campus (San Luis, Argentina). The birds were housed individually in cages (0.50 m×0.30 m×0.35 m) indoors under relatively constant environmental conditions (25.2±0.3°C, relative humidity of 50 ± 9%)
on a photoperiod of 14:10 h (L:D) with ad libitum water and food (mix of seeds, vitamins and minerals, Gausch SA Bahia Blanca). Animals were acclimatized to laboratory conditions for at least 15 days prior to use in experiments. After experiments the birds were released. The routine animal care procedures and experimental procedures used in this study were reviewed and approved by the University of Wisconsin Research Animal Resources Center.

Test probe molecules

Carbohydrates were purchased from Sigma Chemicals, St. Louis: L-arabinose (C5H10O5, MW = 150.11), L-ribose (C5H10O5, MW = 162.16), D-glucose (C6H12O6, MW = 180.16), and lactulose (C12H22O11, MW = 342.33). The aqueous diffusivities were assumed to decline with increasing MW
\(^{1/2}\) (Smudler and Wright 1971).

Experimental treatments

The night before a trial, food was removed. In the morning, birds were randomly assigned to one of three treatments: control (treatment C), mannitol (no nutrient) in the gut; glucose (treatment G), glucose solution in the gut; and food (treatment F), food in the gut. In a fourth kind of trial, probes were injected into the peritoneal cavity. Birds in treatment C and treatment G were provided water but no food during the 1st h after lights turned on and then were gavaged three times (every 20 min) with a 500 mg solution containing 75 mM NaCl and either 210 mM D-mannitol (treatment C) or 110 mM D-glucose + 100 mM D-mannitol (treatment G). Gavage was performed in 
\(\leq 30\) s using a cannula with a diameter of 3 mm, inserted through the vent into the stomach without anesthesia. Birds in treatment F were provided water and the starch-containing diet for the 2 h after lights turned on and then were gavaged twice with probes prepared in water. For the measurement period beginning 2 h after lights off, on birds in treatments C, G, and F were gavaged with 500 mg test solution containing four probes (20 mM each of L-arabinose, L-ribose, D-glucose, D-mannitol) and 30 mM lactulose, 75 mM NaCl, 10 mM potassium ferrocyanide, and either 75 mM mannitol (treatment C) or 75 mM D-glucose (treatment G and treatment F, respectively). We initially included the nonabsorbable marker ferrocyanide in order to calculate digesta residence times (Cheek and Oldham 1991) but decided later to omit this measurement in these particular birds, although we do report data on other birds measured in this way. Birds injected received 150 mg test solution containing the four probes at each of a concentration of 90 mM. Osomatic pressures of solutions were measured (Wescor VAPRO 3550) prior to administration and averaged 546 ± 1 mmol for treatments C, G, F, and 371 ± 1 mmol for injection trials.

During the 100 min after gavage or injection, a series of blood samples (40 ml) were collected from the brachial vein with heparinized capillary tubes. Five to six blood samples (240 at total, which accounts for <10% of total blood volume; Stangel 1986) were collected. The samples were centrifuged for 3 min at 10,000 rpm in a hematocrit centrifuge (Eavour model VT-1224) and the plasma was separated and stored at 0°C.

Pharmacokinetic calculation of absorption

The plasma concentrations C (units, mg probe g

\(^{-1}\) plasma) were plotted as a function of sample time t (min). The amounts of probes absorbed were calculated from areas under the post-absorption and post-injection plasma curves (AUC = area under the curve of plasma probe concentration vs. time, in units of mg g

\(^{-1}\) plasma). This simple method does not require assumptions about pool sizes and (or 2- or 3-pool) or kinetics (e.g., 1st order) (Welling 1986). Fractional absorption (F), also called bioavailability, was calculated as:

\[
F = \frac{\text{AUC}_{\text{gavage}}}{\text{AUC}_{\text{injection}}} = \frac{\text{AUC}_{\text{gavage}}}{\text{AUC}_{\text{injection}}}
\]

Following typical procedures in pharmacokinetics (Welling 1986), the area from t = 0 to t = x min (when the final blood sample was taken) was calculated using the trapezoidal rule. The area from t = x min to t = ∞ was calculated as:

\[
\text{AUC}_{\text{gavage}} = \int C(t) dt
\]

The total AUC

\(^{\text{gavage, t = x}}\) was obtained by summing the two areas. The parameter

\(k_{\text{el}}\) (in units of min

\(^{-1}\)) is the elimination constant for removal of the probe from plasma, which was estimated by regressing (least squares regression, SYSTAT, Wilkinson 1992) the last three log-transformed plasma concentrations C (units, mg probe g

\(^{-1}\) plasma) against t and calculating the slope. In analogous fashion the data for the injected birds were extrapolated back to t = 0 using the slope of the first three log-transformed plasma concentrations.

Statistical analyses

Numerical results are given as means ± SEM (n = number of animals unless otherwise indicated). The values of F for the four probes were arcsine-square-root transformed prior to statistical comparisons. ANOVA, using the general linear model in SYSTAT (Wilkinson 1992), was used to test for differences among probes and treatments. For most birds we had measures for all four probes measured simultaneously for a given treatment, and for all birds we had measurements in all three treatments (C, G, F). Therefore, one statistical procedure was to make treatment a factor and probes a repeated-measure within the bird, and a second statistical procedure was to make treatment a factor and treatments a repeated-measure. There was no procedure that we knew of that would permit comparisons in a single ANOVA with both treatment and probes as repeated measures. The F statistics of these and other analyses of variance are presented in the text with the relevant degrees of freedom as subscripts.

Results

Elimination of probes following injection

Probes in the circulatory system were cleared rapidly, with apparently 98% of elimination occurring over the course of a typical 2-h trial (Fig. 1). Nonlinear fits of these data to a mono-exponential elimination model gave values of

\[r^2 > 0.99\] for every probe, which supports our method of estimating the residual AUC past
Fractional absorptions ($F$) were calculated using information in both Figs. 1 and 2. They differed significantly according to treatment (repeated measures on arcsin-square-root transformed values $F_{2,30}=4.56$, $P=0.014$), irrespective of the particular probe (i.e., no significant interaction; $F_{3,30}=0.33$, $P>0.9$; Fig. 3). Fractional absorption averaged over all probes was 0.59±0.12 for the house sparrows fed or gavaged with D-glucose and 0.62±0.09 for the sparrows gavaged with mannitol. However, there were significant differences among the probes (repeated measures on arcsin-square-root transformed values $F_{5,60}=33.2$, $P<0.001$), irrespective of the particular treatment (i.e., no significant interaction; $F_{5,60}=0.3$, $P>0.9$). Fractional absorption averaged over all treatments was 0.61±0.09 for arabinose, 0.64±0.13 for rhamnose, 0.46±0.08 for perositol, and 0.15±0.03 for lactulose.

Some of the 75% overall decline in absorption (i.e., 0.64–0.15) with increasing probe MW might be expected simply on the basis of differences in their free aqueous diffusivities. However, even when normalized to MW$^{1/2}$ to approximately correct for this (Smulders and Wright 1971), the overall decline in absorption with increasing probe size was still 60% (Fig. 4).

**Discussion**

The understanding and prediction of the extent of intestinal absorption of water-soluble compounds is important in diverse areas such as clinical medicine, nutrition, and ecotoxicology. In the first case, probes such as mannitol and lactulose are routinely used in oral absorption studies to test for intestinal pathologies (Bjornsson et al. 1995; Elia et al. 1987; Maxton et al. 1986; Menzies et al. 1979; Munkholm et al. 1994). In the second and third cases, intestinal permeability is a major determinant of the oral bioavailability of water-soluble nutrients and toxins, and knowledge of structure-absorption relationships facilitates the design of new drugs (Razevik et al. 2000).

The pharmacokinetic technique that we used to measure absorption of carbohydrate probes is widely used to determine the absorption (bioavailability) of drugs and toxins and we have used it before (Caviedes-Vidal and Karasov 1996; Cediack et al. 2001). The approach involves oral gavage of probes that are non-metabolizable (Caviedes-Vidal and Karasov 1996; Cediack et al. 2001; Dahlqvist and Gryboski 1965; Hamilton et al. 1987) and lack affinity for mediated uptake mechanisms (Cediack et al. 2001; Fu et al. 2000; Hamilton et al. 1987). After gavage we sampled blood, which is required when working with non-mammalian vertebrates in whom urinary wastes are pooled with fecal wastes. A simpler approach in mammals is to feed the probes and recover them in urine, and this is the method for clinical tests and scientific studies of intestinal permeability in humans and rats that we reviewed (Table 1). Estimates of oral absorption should take
account of possible differential recovery of probes when injected, and such measures have been taken in our studies with sparrow and about half the studies in humans and rats (Table 1) and recoveries of mannitol and lactulose are uniformly high (Riviere 1999; Sharpe and Yu 1999). An important virtue of the pharmacokinetic method is that it can provide measures of absorption under fairly natural conditions, which is important as some studies suggest that the paracellular permeability is affected by surgical manipulations (Uhrig and Kimura 1995b).

We predicted and observed that absorption of probes by sparrow would decline with increasing size of test probe and would be enhanced when measured in the presence of luminal nutrients (Fig. 3). In the sections below we compare our results with related findings in mammals and discuss how the patterns across probes and even differences among species can be usefully interpreted by applying the theoretical understanding of how water-soluble molecules cross epithelia.

Molecular size discrimination

House sparrows absorb water-soluble probes to a greater extent than humans and much more than laboratory rats, but all species show the pattern of reduced absorption with increasing molecular size (Table 1). The differences in absorption between species are in most cases statistically significant. In comparing, for example, many measures in humans and rats by different laboratories, one finds that fractional absorption by humans of orally administered mannitol (0.26 ± 0.03, n = 4) is about seven times greater than that by rats (0.04 ± 0.1, n = 3), and that absorption of lactulose by both species is lower than for mannitol (respectively, 0.007 ± 0.003, n = 7 and 0.013 ± 0.008, n = 2) and does not differ between the species (2-way ANCOVA on the arcsin-square-root transformed values; $F_{1,12} = 25.3$, $P = 0.001$ for species, and $F_{1,12} = 34.6$, $P < 0.001$ for the species-probe interaction). Mean probe absorption by house sparrows falls outside and above the 95% confidence interval for humans for mannitol, lactulose, and rhamnose, and the duplicate measures of arabino absorption in house sparrows fall well above the single measure in humans (Table 1).

As reviewed in the Introduction, it has been presumed that these water-soluble molecules permeate across the small intestinal mucosal epithelium primarily through the paracellular pathway (Madara and Pappenheimer 1987; Pappenheimer 1987; Pappenheimer and Reiss 1987; Powell 1987). If so, the differences between species and probes might be explicable in the context of the Kedem–Katchalsky equation (Kedem and Katchalsky 1958) that describes the contributions of
diffusive and convective flux to total solute flux \( J_s \) through porous epithelia:

\[
J_s = [(C_1 - C_2)DA/L] + [(1 - \sigma)J_{s0}(C_1 + C_2)/2]
\]

where \( C_1 \) and \( C_2 \) are concentrations of solute at the two ends of the channel, \( D \) is the diffusion coefficient, \( AL \) is the cross-sectional area per unit path length of the channel, \( \sigma \) is the coefficient of solvent drag, and \( J_s \) is flow of solvent. Modifications have been made to account for sieving according to molecular size (molecular radius, \( a_0 \), has been the parameter of choice) for both the diffusive (first) and the convective (second) terms in Eqn. 1 in the course of developing a theory for transport through porous membranes or epithelia including capillary walls (Pappenheimer 1953), the glomerulus (Renkin and Gilmore 1973), and intestinal epithelia (Knipp et al. 1997; Leathy et al. 1994; Pappenheimer and Reiss 1987).

Essentially, a dimensionless sieving function \( F(a_0/\sigma) \) (where \( a_0 \) = pore radius; Crone and Christensen 1979; Curry 1984) has been incorporated into the coupling coefficients of both the diffusive term and the convective term (1-\( \sigma \)). The function, which takes values between 0 and 1, predicts the hindrance of a pore of size \( r_p \) to a molecule of size \( a_0 \) (Pappenheimer and Reiss 1987) omitted the diffusive component of Eqn. 1 because it was insignificant in comparison with the convective component (see also Leathy et al. 1984) and presented a revised equation along with tabular values (Curry 1984) for \( \sigma > 1 \):

\[
J_{s0}/C_1 = (1 - \sigma)F[a_0/(1 + (1 - \sigma)/(1 + \sigma))]^2/2
\]

where \( f_s \) is the fraction of total fluid absorption that takes place through paracellular channels. A notable result of these modeling efforts is the opportunity to predict how probe clearance \( (J_s/C_1) \) might vary if effective pore radius or solvent flow vary, as might occur within and between species.

Using physiologically reasonable values for \( J_s \) (summarized in Pappenheimer 1998), and setting \( f_s = 0.5 \) (Pappenheimer and Reiss 1987), we find over the size range of molecules that we studied that clearance is a sharply declining, sometimes sigmoidal function of \( a_0 \), unless \( r_p \) is very high (Fig. 5a). The rationale for modeling very large \( r_p \) (up to 50 Å) is that the tight junction appears in electron micrographs as a series of close cell-cells contacts formed by transmembrane protein particles that are about 100 Å across, spaced at a center-to-center distance of 180 Å (Anderson 2001), thereby possibly creating a maximum sieve or effective pore size of 80 Å. The space could be smaller if the protein particles are interdigitated like the two halves of a zipper (Anderson 2001). However, the empirical data (Fig. 5d), when compared with the modeling results for varying \( r_p \) (Fig. 5a) do not seem consistent with such a large effective pore size but seem consistent instead with an effective pore size in the range of 5-10 Å. Over that range of pore sizes (Fig. 5b, c), changes in \( J_s \) greatly, and in \( r_p \) to a lesser extent, change the magnitude of clearance and the slope of the relation between clearance and \( a_0 \).
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<th>Species</th>
<th>Mode of Administration</th>
<th>Fluid Sampled</th>
<th>Measured Fractional Absorption</th>
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*a* these are mean values measured in the absence of luminal nutrients and so they are lower than the means over all treatments that are reported in Results.

In light of this model, and assuming that differences in fractional absorption in oral bioavailability studies correspond to differences in clearance (discussed below), it seems possible to make some tentative deductions. First, it does not seem possible that pore size, $r_p$, equals 50 A or even 20 A in the human or rat because, at any reasonable value of $J_T$, the theoretical slopes over the size range of molecules that have been studied are too shallow (Fig. 5d). For human Caco-2 epithelial cells, estimated $r_p$ was 5.2–12.9 A (Adson et al. 1994, Kaupp et al. 1987) using Stokes-Einstein estimates of molecular radii of test probes, which just exceeds the Stokes-Einstein estimate for the molecular radius of lactulose (5.1 A Ghandehari et al. 1997). Second, it does seem possible, however, that effective pore size, $r_p$, is greater in house sparrows than in the mammals because fractional absorption of large-sized lactulose by house sparrows is about an order of magnitude higher than the very low values in the mammals (Table 1). But it is also conceivable that this difference might result from the same $r_p$ in conjunction with a very much higher value of $J_T$. Third, the difference between the two mammal species may relate more to differences in $J_T$, than $r_p$, because the similarity in absorption between humans and rats for large-sized lactulose in conjunction with the seven-times-higher absorption in humans than rats of smaller sized mannitol implies that $J_T$ is higher in humans. It is known that fluid absorption increases in the presence of glucose to a maximum when luminal glucose concentrations are in the range 25–100 mM, but at all concentrations the rates in human jejunum are about ten times higher than in rat jejunum (e.g., Fig. 6 in Pappenheimer 1998). The data in Table 1 were collected in intact animals without exogenously supplied luminal glucose, but there is probably always some glucose diffusing in from plasma (5–6 mM in humans and rats). The 3-fold higher plasma glucose in birds than in mammals (Altman and Dittmer 1978) might contribute towards higher baseline fluid flux and partially explain the apparently higher paracellular absorption in sparrows in Table 1.

We have assumed that differences in fractional absorption in oral bioavailability studies correspond to differences in clearance ($J_T/C_I$). This seems reasonable for cases for oral administration of a mix of probes in a volume ($V$) to a single animal because all probes are potentially subject to the same intestinal surface area ($S_{ma}$ cm$^{-2}$) and the same contact time ($T$) between solution and intestinal area. We focus on those variables because ($J_T/C_I$) becomes the unit-less fractional absorption $F$. Even between species with very different body mass (M; e.g., rat vs. human) it seems reasonable to suppose that differences in fractional absorption in oral bioavailability studies correspond to differences in per unit area clearance. If the two species are given oral volumetric doses equivalent to some constant proportion of their body mass (i.e., $V_{Ma}M^{-1}$), then considering that $S_{ma}M^{0.25}$ and $V_{Ma}M^{1.25}$ (Karassov and Hume 1997), $S_{ma}/V$ will approximate a constant ($C_{Ma}M^{-0.75}$) and thus the differences in ($J_T/C_I$) will translate to differences in $F$.

The above analysis and the comparisons in Fig. 5 suggest that it is most profitable to investigate passive absorption of water-soluble solutes by measuring oral bioavailability of a series of probes up to the size where absorption no longer occurs. It is to be expected that absorption vs. molecular size may appear to be biphasic
Fig. 5A-D Patterns of water-soluble probe clearance \( (C_p) \) by solvent drag in the intestine as a function of estimated probe molecular radius \( (r_p) \) and different volumetric flow rates \( (J_v) \), along with empirical data on fractional absorption as a function of \( n_a \). The effect of different pore sizes \( (r_p = 5, 10, 20, \text{and } 50 \text{ Å}) \) on predicted clearance is illustrated in A (above left), with \( J_v \) set at 2 ml h\(^{-1}\) cm\(^{-2}\). The effect of different \( J_v \) (in ml h\(^{-1}\) cm\(^{-2}\)) on predicted clearance is shown for pore size of 5 Å (B above right) and 10 Å (C down left). \( J_v \) was set at 1 μmol/ ml, so that over the range of \( r_p \) the value of \( J_v/C_p \) (in ml h\(^{-1}\) cm\(^{-2}\)) can range from 0 to 1.0 (see Fig. 2). See the text for other model parameters. The fractional absorption values \( (F) \) in D (down right) are those reported in Table 1 for probes that were measured in all the species and the estimated molecular radii are from Bjarnason et al. (1995). The lines through the values for each species were fitted by the LOESS procedure in SYSTAT (Wilkinson 1992).

because of the sigmoid relationship. Interpretation of such data as reflecting passage through pores of two or more sizes (e.g., Hamilton et al. 1987) fails to take advantage of the simpler explanation of molecular sieving through a single effective pore. On the other hand, the cautionary warning about interpreting experimental phenomena in physical terms (Friedman 1987; Schultz 1980) bears repeating: the "pores" may be tortuous channels not necessarily characterized by a single radius, and characterizing the size and critical features that determine the absorption of probe molecules is not a trivial exercise (Bjarnason et al. 1995; Ghandehari et al. 1997; Raevsky et al. 2000). For example, mannitol has been considered to have both a smaller (Bjarnason et al. 1995; Hollander et al. 1988) and a larger (Hamilton et al. 1987) molecular radius than rhamnose. The estimation of effective pore size may be more sensitive to small uncertainties in the assumed molecular radii of probes than to experimental variability in the measurement of absorption (Adson et al. 1994).

In the case of the house sparrows, we would predict that the molecular size at which absorption becomes zero would significantly exceed the upper limit in humans, which apparently lies just above the molecular radius of lactulose. Such a difference seems important insofar as it partly determines the size range of water-soluble xenobiotics able to be absorbed. As an example, one both predicts (from Fig. 5d) and finds that the oral bioavailability of water-soluble drugs is greater in humans than in rats (He et al. 1998). By analogy, we might test for greater exposure to water-soluble toxins in sparrows (or birds more generally) than in rats and humans (or mammals more generally). Finally, differences in apparent pore size between species as suggested here and elsewhere (He et al. 1998) invite further comparative study on the qualities of the absorptive path(s) that cause them.
Enhanced absorption in the presence of luminal nutrients

Our finding of enhanced absorption in all the probes when measured in the presence of luminal glucose or food as compared with luminal mannitol (Fig. 3) complements our other findings of enhanced absorption of L-glucose under similar conditions (Chang and Karasov 2001; J.G. Chudack, unpublished observations). In those studies, fractional absorption of L-glucose was significantly higher when gavaged to fed rather than fasted birds, or when gavaged to fasted birds in solution with 200 mM D-glucose (+ 80 mM NaCl) than when gavaged with 200 mM mannitol. The difference did not appear to be due to differences in transit through the intestine, as mean retention time of the water-soluble impermeant marker ferrocyanide was 76 ± 5 min in fed house sparrows and 89 ± 6 min in fasted house sparrows (P < 0.3). Besides fractional absorption, we also estimated apparent rates of absorption. The absorption rate constant, Kₘ, for L-glucose determined by curve stripping was 133% higher in birds gavaged with 200 mM D-glucose than when administered with 200 mM mannitol (P = 0.009). With those results we rejected the suggestion (Schwartz et al. 1995) that increased fractional absorption of L-glucose in the presence of luminal nutrients occurs because a slow rate of absorption is extended over a longer time period or a greater length of the intestine. Indeed, it took less (not more) time for birds in the glucose group to take up 99% of whatever probe was absorbed, compared with the mannitol group (30 min vs. 65 min, respectively).

Those results and the results in Fig. 3 are consistent with the hypothesis that small, water-soluble compounds are absorbed faster and to a greater extent when there are nutrients (e.g., sugar, amino acids) in the gut. The mechanism(s) is not known, but might be increased solvent drag (increased Jₑ) and/or cytoskeletal contractions (Madara and Pappenheimer 1987; Madara et al. 1986, 1998; Pappenheimer 1987; Pappenheimer and Reiss 1987) or protein strand alterations that alter the tight junction effective pore size. With the data at hand we do not see a way to distinguish between these explanations. We tried to test the second explanation by measuring absorption of a series of probes of increasing size in the presence and absence of luminal nutrients, but we did not extend the series to a large enough size. We plan future experiments along these lines in both mammals and birds.

Our findings of enhanced absorption in the presence of luminal nutrients are consistent with similar findings in some, but not all, studies with humans and laboratory animals. For example, activation of intestinal Na⁺-nutrient cotransport increased paracellular movement of inert tracers in cultured monolayers (Fricker and Drew 1995; Turner et al. 1997), isolated rodent intestine (Pappenheimer 1987; Pappenheimer and Volpp 1992), and in rodents in vivo (Pappenheimer 1987; Perez et al. 1993; Sadowski and Meddings 1993; See and Bss 1995). In humans, it was recently reported that the bioavailability (i.e., absorption) of orally administered creatinine (a putative paracellular probe) was significantly higher when administered in the presence of 277 mM D-glucose compared with the same concentration of mannitol (Fₚ = 0.55, and Fₚ < 0.35, respectively; Turner et al. 2000). On the other hand, in another recent study, L-glucose absorption was low and not enhanced in unanesthetized dogs infused with solutions with high D-glucose concentration (Lane et al. 1999). Furthermore, enhancement by luminal nutrients was not observed in some studies with rats (Schwartz et al. 1995; Uhung 1998), though the failure to observe enhanced lactulose absorption (O'Raorke et al. 1995) can probably be discounted in light of the considerations above. It is possible that the contrasting findings on enhancement of passive absorption by luminal nutrients could be resolved if uniform methods were applied across different species. However, we are also becoming more accepting of the notion that there might be significant differences between species in the magnitude and control of paracellular transport. In either event, we think that a systematic study across several species using uniform methodology would be an important contribution toward resolving this issue.

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