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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, perseitol, lactulose; MW 150.1–342.3 Da) were gavaged in nonanesthetized House sparrows (*Passer domesticus*), or injected into the pectoralis, and serially measured in plasma. Bioavailability was calculated as F = AUC 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 sieve · Paracellular absorption · Small intestine

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Abbreviations a_e molecular radius · AUC area under the curve · C_1/C_2 concentrations of solute at two ends of the channel · F fractional absorption · *PEG* polyethylene glycol · J_s total solute flux · J_v flow of solvent · r_e pore radius · S_m intestinal surface area · *treatment C* control treatment · *treatment F* food treatment · *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; Chediack et al. 2001; Hamilton et al. 1987; Karasov and Cork 1994), lipid-insoluble octapeptides (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 (Madara 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. Aqueous pores are thought to exist within the paired strands (Tsukita 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; Meehye 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 (''sieving''; 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 1993), medium chain fatty acids (Lindmark et al. 1998) and natural toxins such as capsianoside, 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 (esophagus, 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 *does* occur. Second, there is evidence that approaches not relying on intact animals may introduce artifacts (e.g., Uhing 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 Hollander 1987; He et al. 1996; 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; Leahy 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; Chediack 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 Bahía Blanca). Animals were acclimated 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 ($C_5H_{10}O_5$, MW = 150.13), L-rhamnose ($C_6H_{12}O_5$, MW = 164.2), perseitol ($C_7H_{16}O_7$, MW = 212.2), and lactulose ($C_{12}H_{22}O_{11}$, MW = 342.3). The aqueous diffusivities were assumed to decline with increasing $MW^{1/2}$ (Smulders 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; food (treatment F), food in the gut. In a fourth kind of trial, probes were injected into the pectoralis muscle. 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 ≤ 30 s using a cannula with a blunt edge inserted deeply through the esophagus 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. Birds injected with probes were provided with only water. For the measurement period beginning 2 h after lights 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-rhamnose, perseitol 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 (Chediack et al. 2001) 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 each at a concentration of 90 mM. Osmotic pressures of solutions were measured (Wescor VAPRO 5520) prior to administration and averaged 346 ± 1 mosmol for treatments C, G, F and 371 ± 1 mosmol for injection trials.

During the 100 min after gavage or injection, a series of blood samples (40 µl) were collected from the brachial vein with heparinized capillary tubes. Five to six blood samples (240 µl 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.

Analysis of probes

Plasma proteins were precipitated using 10% $ZnSO_4$ and acetonitrile (Merck, Germany HPLC quality; Lam and Malikin 1989). We gently agitated the tube for a few seconds and centrifuged at 4000 rpm for 3 min. The pellet was discarded and the supernatant was vacuum-dried. With the dried sample we performed a syrilation reaction, which makes the compound volatile for gas chromatography, using 98% chlorotrimethylsilane (Aldrich, Milwaukee, USA) and hexamethyldisilazane (Sigma Chemicals, St. Louis) in pyridine (Merck, Germany). The syrilation was performed 40 min before injection into the gas chromatograph (Hewlett-Packard model 5890 Series II plus), which was fitted with an HP 5 column (5% phenyl-methylsilicone) and a mass detector (Hewlett-Packard model 5972). The analyses and quantification of the sugars were carried out using the ChemStation software provided with the equipment. To estimate L-arabinose we integrated two peaks, at 8.4 min and 9.1 min; for L-rhamnose we used two peaks at 8.7 min and 10.15 min; for perseitol, one peak at 17.1 min; for lactulose, two peaks at 21.7 min and 21.98 min. The software integrates the peaks and gives arbitrary units. To determine the concentrations of the unknown samples, we made calibration curves for each probe every day. The lowest level of detection was 1 ppb.

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 min g^{-1} plasma). This simple method does not require assumptions about pool sizes (e.g., 1- or 2- pools) or kinetics (e.g., 1st order) (Welling 1986). Fractional absorption (F), also called bioavailability, was calculated as $F = (\text{AUC by gavage}/\text{dose}_{\text{gavage}})/(\text{AUC by injection}/\text{dose}_{\text{injection}})$. 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 $t = \infty$ was calculated as $AUC^{x \rightarrow \infty} = (C \text{ at } t = x)/k_{el}$. The total $AUC^{0 \rightarrow \infty}$ was obtained by summing the two areas. The parameter k_{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 arcsin-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 probe 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 ≥90% 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

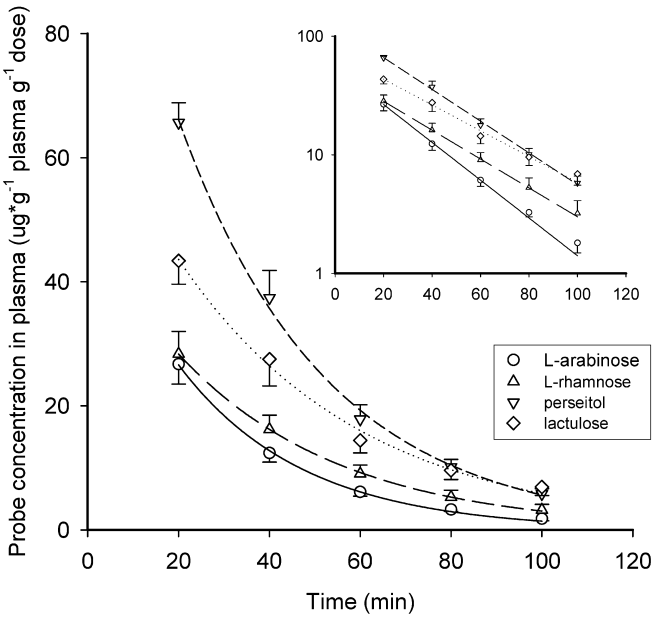

Fig. 1 Carbohydrate probes injected into house sparrows were cleared from the blood rapidly in a mono-exponential pattern. Probes were L-arabinose (*circle*, *solid line*), L-rhamnose (*triangle*, *large dashed line*), perseitol (*inverted triangle*, *small dashed line*), and lactulose (*diamond*, *dotted line*). The *lines* in the *larger figure* were fitted by a log smoothing routine. *Inset* shows the same data for each probe on a semi-logarithmic plot, where for each probe the least-squares linear fit was very good (all $r^2 > 0.99$). The data points are means ± SEM ($n = 12$–14 sparrows)

100 min using the apparent elimination rate constants (see Materials and methods). As shown in the next section, the elimination rates in these injection/elimination experiments did not differ significantly from the terminal slopes in the post-absorption plasma curves. However, there were significant differences among the probes themselves, with rates higher for arabinose (-0.035 ± 0.005 min^{-1}) and perseitol (-0.035 ± 0.004 min^{-1}) than for rhamnose (-0.024 ± 0.004 min^{-1}) and lactulose (-0.027 ± 0.005 min^{-1}) ($F_{3,231} = 2.8$, $P = 0.04$).

Absorption of probes following gavage

Plasma probe concentrations were elevated 7 min post-gavage (the first sampling time) and peaked at 15 min post-gavage (Fig. 2). Subsequently, plasma probe concentrations declined in a mono-exponential fashion as occurred for elimination of probes following their injection (see Fig. 1). Indeed, comparison of the terminal slopes based on the last three log-transformed plasma concentrations of the post-gavage and post-injection trials found the slopes not significantly different for arabinose ($F_{3,145} = 1.3$; $P = 0.28$), rhamnose ($F_{3,142} = 0.46$, $P > 0.7$), perseitol ($F_{3,144} = 0.7$; $P > 0.5$), and lactulose ($F_{3,140} = 0.9$; $P > 0.4$). Thus, whether the gut contained either food (injection trial, one of the gavage trials) or solution with mannitol or glucose did not appreciably change the rate of elimination of each probe from the body.

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 $F_{2,70} = 4.56$, $P = 0.014$), irrespective of the particular probe (i.e., no significant interaction; $F_{6,70} = 0.33$, $P > 0.9$; Fig. 3). Fractional absorption averaged over all probes was 0.59 ± 0.12 for the house sparrows fed or gavaged with D-glucose and 0.42 ± 0.09 for the sparrows gavaged with mannitol. However, there were significant differences among the probes (repeated measures on arcsin-square-root transformed values $F_{3,69} = 33.2$, $P < 0.001$), irrespective of the particular treatment (i.e., no significant interaction; $F_{6,69} = 0.3$, $P > 0.9$). Fractional absorption averaged over all treatments was 0.61 ± 0.09 for arabinose, 0.64 ± 0.13 for rhamnose, 0.46 ± 0.08 for perseitol, and 0.15 ± 0.05 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 (Bjarnason 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 (Raevsky 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; Chediack et al. 2001). The approach involves oral gavage of probes that are non-metabolizable (Caviedes-Vidal and Karasov 1996; Chediack et al. 2001; Dahlqvist and Gryboski 1965; Hamilton et al. 1987) and lack affinity for mediated uptake mechanisms (Chediack 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

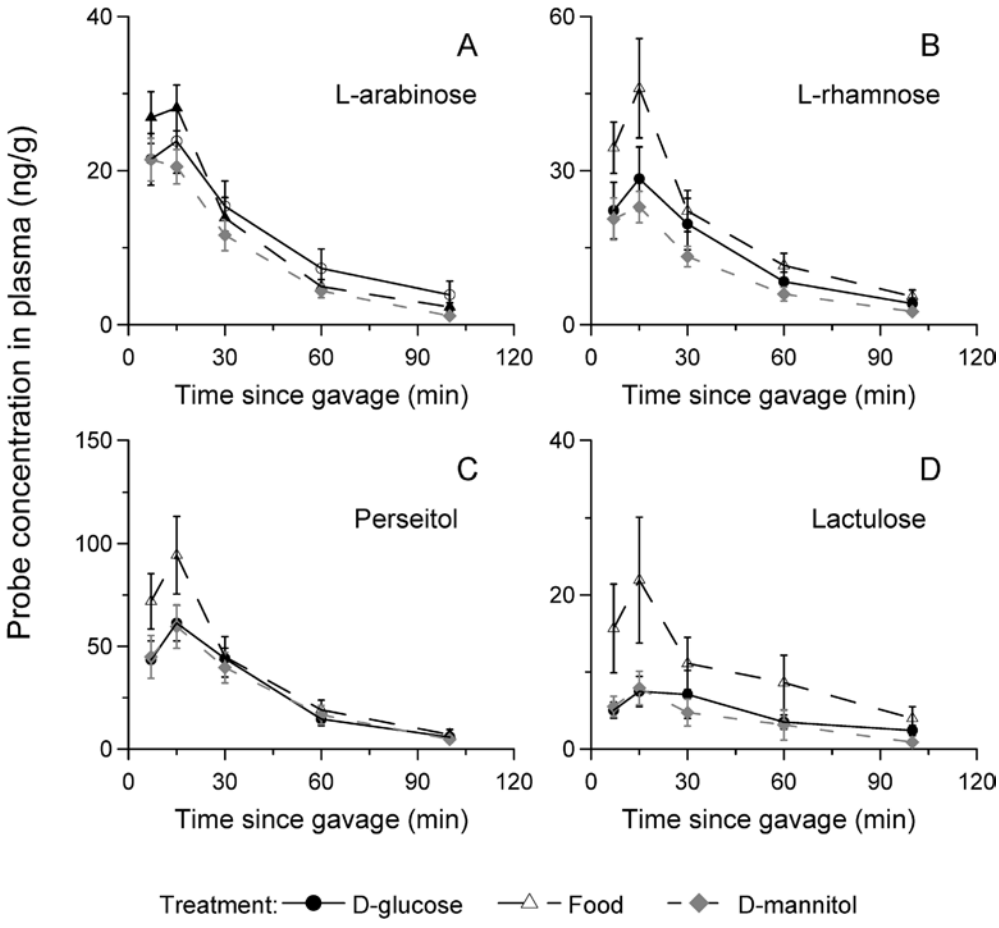

Fig. 2A–D Carbohydrate probes fed by gavage to house sparrows appeared in plasma by 7 min post-gavage (the first sampling time), peaked at 15 min post-gavage, and were subsequently cleared from the plasma rapidly in a mono-exponential pattern. Four probes were administered simultaneously but are plotted separately: **A** L-arabinose, **B** L-rhamnose, **C** perseitol, **D** lactulose. They were administered to sparrows in three treatment groups designated as "D-mannitol" (mannitol solution in the gut; *filled diamond* with *smaller dashed line*), "D-glucose" (glucose solution in the gut; *filled circles* with *solid line*), or "Food" (food in the gut; *unfilled triangle*; *larger dashed line*). All sparrows received the same dose by mass (503.8 ± 5.4 mg) and the concentration was 30 mM for lactulose and 20 mM for the other three probes. The data points are means ± SEM ($n = 12$–14 sparrows)

account of possible differential recovery of probes when injected, and such measures have been taken in our studies with sparrows and about half the studies in humans and rats (Table 1) and recoveries of mannitol and lactulose are uniformly high (Riviere 1999; Shargel 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 (Uhing and Kimura 1995b).

We predicted and observed that absorption of probes by sparrows 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 ± 0.01, $n = 3$), 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 arabinose 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; Pappemheimer 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

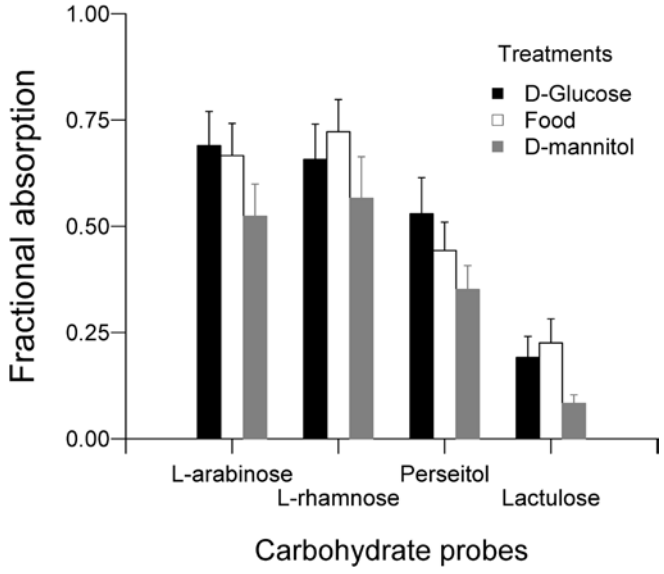

Fig. 3 Water-soluble probe absorption by house sparrows depended on molecular weight and increased when measured in the presence of nutrients. The calculated fractional absorptions are plotted according to increasing molecular weight (MW) of the probes (left to right; MW in Daltons; L-arabinose MW 150.1, L-rhamnose MW 164.2, perseitol MW 212.2 and lactulose MW = 342.3). The probes were administered simultaneously to sparrows in three treatment groups designated as "D-mannitol" (no nutrient in the gut; *gray bars*), "D-glucose" (glucose solution in the gut; *black bars*), or "Food" (food in the gut; *unfilled bars*). Means ± SEM ($n = 12$–14 sparrows) are shown. See text for statistical comparison

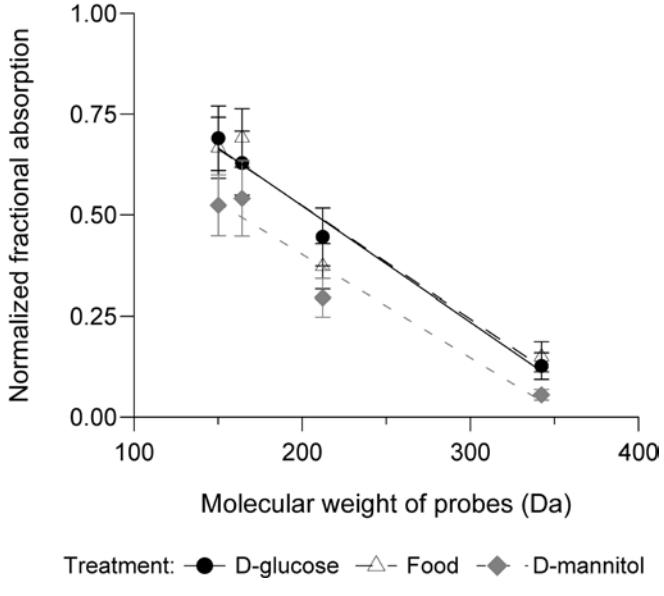

Fig. 4 Fractional absorption declined with increasing molecular size even when corrected for differences in aqueous diffusivity of the probes. Each point is the mean value for probe *i* from Fig. 3 divided by $[MW^{1/2}{}_{arabinose}]/[MW^{1/2}i]$ to correct for differences in diffusivity (Smulders and Wright 1971). The three treatment groups are designated as "D-mannitol" (no nutrient in the gut; *gray diamond* with *smaller dashed line*), "D-glucose" (glucose solution in the gut; *filled circles* with *solid line*), or "Food" (food in the gut; *unfilled triangle*; *larger dashed line*). The *lines* are least squares regressions

diffusive and convective flux to total solute flux (J_s) through porous epithelia:

$$J_s = [(C_1 - C_2)DA/L] + [(1 - \sigma)J_v(C_1 + C_2)/2] \quad (1)$$

where C_1 and C_2 are concentrations of solute at the two ends of the channel, D is the diffusion coefficient, A/L is the cross-sectional area per unit path length of the channel, 1-σ is the coefficient of solvent drag, and J_v is flow of solvent. Modifications have been made to account for sieving according to molecular size (molecular radius, a_e, 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; Leahy et al. 1994; Pappenheimer and Reiss 1987). Essentially, a dimensionless sieving function $F(a_e/r_e)$ (where r_e = 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-σ). The function, which takes values between 0 and 1, predicts the hindrance of a pore of size r_e to a molecule of size a_e. Pappenheimer and Reiss (1987) omitted the diffusive component of Eqn. 1 because it was insignificant in comparison with the convective component (see also Leahy et al. 1984) and presented a revised equation along with tabular values (Curry 1984) for (1-σ):

$$J_s/C_1 = (1 - \sigma)fJ_v[1 + (1 - \sigma)/(1 + \sigma)]/2 \quad (2)$$

where f 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_v (summarized in Pappenheimer 1998), and setting $f = 0.5$ (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_e, unless r_e is very high (Fig. 5a). The rationale for modeling very large r_e (up to 50 Å) is that the tight junction appears in electron micrographs as a series of close cell-cell 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_e (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. 5 b, c), changes in J_v greatly, and in r_e to a lesser extent, change the magnitude of clearance and the slope of the relation between clearance and a_e.

Table 1 Absorption of water-soluble probes in sparrows, humans, and rats measured in absence of luminal nutrients

Species	Mode of Administration		Fluid Sampled		Measured Fractional Absorption				Reference
	Oral	Inject	Urine	Blood	Arabinose	Mannitol	Rhamnose	Lactulose	
Sparrow	x	x		x	0.61[a]		0.59[a]	0.09[a]	This study
Sparrow	x	x		x	0.69	0.63			Chediack et al. 2001
Human	x		x			0.272	0.116	0.00119	Munkholm et al. 1994
Human	x		x		0.175				Lobley et al. 1990
Human	x	x	x			0.19		0.0041	Elia et al. 1987
Human	x	x	x			0.342	0.163	0.015	Delahunty and Hollander 1987
Human	x		x			0.215		0.0047	Bijlsma et al. 1995
Human	x	x	x				0.189	0.0042	Maxton et al. 1986
Human	x	x	x				0.154	0.0189	Menzies et al. 1979
Human	x		x				0.113	0.0041	Dinmore et al. 1994
Rat	x	x	x			0.0625			Schwartz et al. 1995
Rat	x		x			0.019	0.028	0.0044	Delahuntyand Hollander 1987
Rat	x		x			0.0405		0.0204	Bijlsma et al. 1995

[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_e equals 50 Å or even 20 Å in the human or rat because, at any reasonable value of Jv, 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_e was 5.2–12.0 Å (Adson et al. 1994; Knipp 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 Å Ghandehari et al. 1997). Second, it does seem possible, however, that effective pore size r_e 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_e in conjunction with a very much higher value of fJ_v. Third, the difference between the two mammal species may relate more to differences in J_v than r_e, 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_v 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 1974) 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_s/C_1; ml h^{-1} cm^{-2}). This seems reasonable for cases for oral administration of a mix of probes in a volume (V; ml) to a single animal because all probes are potentially subject to the same intestinal surface area (S_m; cm^2) and the same contact time (T; h) between solution and intestinal area. We focus on those variables because (J_s/C_1) S_mT/V 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., $V \propto M^{1.0}$), then considering that $S_m \propto M^{2/3-3/4}$ and $T \propto M^{1/4-1/3}$ (Karasov and Hume 1997), S_mT/V will approximate a constant ($\propto M^{1.0}/M^{1.0}$) and thus the differences in (J_s/C_1) 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

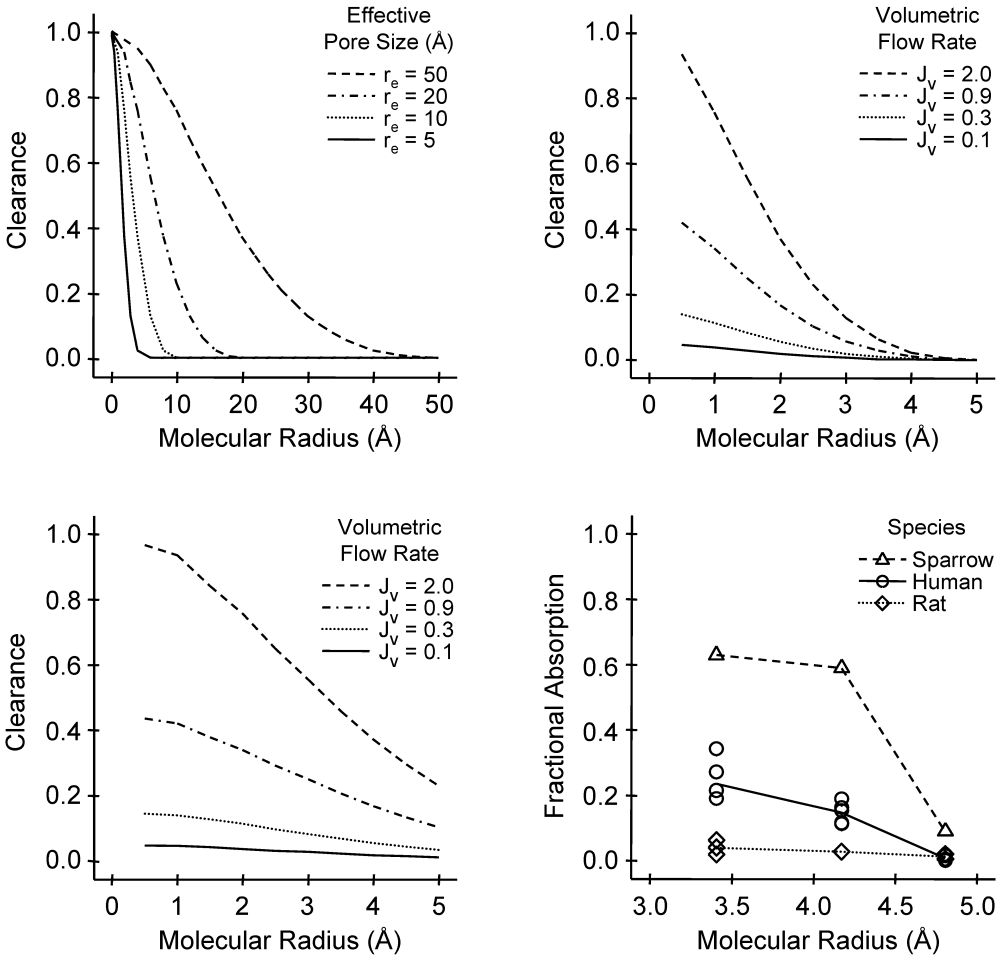

Fig. 5A–D Patterns of water-soluble probe clearance (J_s/C_1) by solvent drag in the intestine as a function of estimated probe molecular radius (a_e) assuming different effective pore sizes (r_e) and different volumetric flow rates (J_v), along with empirical data on fractional absorption as a function of a_e. The effect of different pore sizes ($r_e = 5$, 10, 20, and 50 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 (in ml h^{-1} cm^{-2}) was varied between 0.1 and 2.0 based on measurements in rats and humans summarized in Pappenheimer (1998). C_1 was set at 1 μmol/ml, so that over the range of J_v the value of J_s/C_1 (in ml h^{-1} cm^{-2}) can range from 0 to 1.0 (see Eqn. 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 of 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. Chediack, 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 80 ± 6 min in fasted house sparrows ($P > 0.3$). Besides fractional absorption, we also estimated apparent rates of absorption. The absorption rate constant, K_a, 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 $J_v f$) 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. 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 Bass 1993). 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 ($F = 0.55$, and $F = 0.38$, 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; Uhing 1998), though the failure to observe enhanced lactulose absorption (O'Rourke 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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