PASSIVE ABSORPTION OF HYDROPHILIC CARBOHYDRATE PROBES BY THE HOUSE SPARROW PASSER DOMESTICUS

J. G. CHEDIACK1, E. CAVIEDES-VIDAL1, W. H. KARASOV2,* AND M. PESTCHANKER1

1Departamento de Bioquímica y Ciencias Biológicas, Universidad Nacional de San Luis, 5700 San Luis, Argentina and 2Department of Wildlife Ecology, University of Wisconsin, Madison, WI 53706, USA

*Author for correspondence (e-mail: wkarasov@facstaff.wisc.edu)

Accepted 21 November 2000; published on WWW 1 February 2001

Summary

To evaluate the permeability of the intestine of the house sparrow Passer domesticus to hydrophilic compounds, we applied a pharmacokinetic technique to measure in vivo absorption of two carbohydrate probes, L-arabinose and D-mannitol. Probes were fed or injected, and blood and excreta were subsequently collected and analyzed by gas chromatography/mass spectrometry. Following injection, plasma probe concentration decreased in a log-linear fashion, implying single-compartment, first-order kinetics. Following oral administration, plasma probe concentrations increased, reached a maximum at 10 min and then decreased in log-linear fashion. Mannitol and arabinose absorption were calculated from the areas under the post-absorption plasma curve and the respective distribution spaces and elimination constants. The amounts absorbed increased linearly with the concentration administered (range 1–1000 mmol l⁻¹), implying a passive process. The mouth-to-cloaca retention time of digesta, measured using the non-absorbable compound potassium ferrocyanide, was independent of probe concentration. On average, 69% of the oral dose of probe was absorbed and this was independent of the concentration of probe administered. This paper supports an earlier report of substantial passive glucose absorption in house sparrows and offers a method to study the extent of hydrophilic solute absorption, which has importance for future research in areas as diverse as biomedical, ecological and evolutionary physiology.

Key words: intestinal absorption, passive transport, paracellular transport, Aves, Passer domesticus.

Introduction

The magnitude, mechanism(s) and biological significance of intestinal passive absorption of hydrophilic compounds is currently under debate, much of which has focused around whether the primary path for glucose absorption is transcellular and the mechanism is mediated (Diamond, 1991; Ferraris and Diamond, 1997; Fine et al., 1993; Uhing and Kimura, 1995a; Uhing and Kimura, 1995b) or whether the primary path is paracellular and that the mechanism is passive (Afik et al., 1997; Caviedes-Vidal and Karasov, 1996; Karasov and Cork, 1994; Levey and Cipollini, 1996; Pappenheimer, 1990; Pappenheimer, 1993; Pappenheimer, 1998), but the implications extend well beyond the question of how glucose in a meal is absorbed. The extent of hydrophilic solute absorption has importance for future research in areas as diverse as biomedical, ecological and evolutionary physiology. In the first case, drug therapies rely on accurate information about the pathways of absorption of hydrophilic compounds. In the second case, high intestinal permeability permitting passive absorption might permit hydrophilic toxins to be absorbed from plant and animal material eaten by wild animals (Diamond, 1991). In the third case, attempts to understand the evolution of safety margins for intestinal absorption (Diamond, 1993) may not succeed if passive absorption is important but is not taken into account.

A series of studies on intact birds (Afik et al., 1997; Caviedes-Vidal and Karasov, 1996; Karasov and Cork, 1994; Levey and Cipollini, 1996) indicated that 75–90% of ingested L-glucose was absorbed and excreted in the urine. This was considered evidence of considerable passive glucose absorption because L-glucose is a stereoisomer that is thought not to interact with the D-glucose transporter and is thought to be absorbed passively. However, Schwartz et al. (Schwartz et al., 1995) presented data that they thought cast doubt on this assumption. Considering this, and the fact that all the findings of apparent passive absorption in birds were based on the appearance in the blood of ingested radiolabel, which could dissociate from L-glucose, those studies beg replication using other solutes and other methods.

In this study, we extend the observations of hydrophilic probe absorption in birds by addressing three goals. First, we sought to develop further our in vivo model for studying intestinal absorption. In particular, we wanted to determine the level of absorption of hydrophilic probes administered in solution, because this could be a means later of discriminating the
mechanisms of passive absorption by manipulating the solutions administered orally. Whereas in previous studies we included the probe in the diet and made time-consuming measures under steady-state conditions, in this study we developed short-term procedures involving gavage of solutions and sampling of blood and excreta. Our measurements were made in intact animals for a number of reasons. First, while in vitro methods can demonstrate conditions and mechanisms by which passive absorption can occur, they cannot by themselves demonstrate the mechanisms by which it normally does occur. Much of the argument in the literature about passive absorption is not about whether it occurs, but about its relative magnitude and biological significance in normal animals. Second, there is evidence in the literature that approaches not relying on intact animals may introduce artifacts (Ugolev et al., 1986; Uging and Kimura, 1995a). Thus, progress in understanding passive absorption will be achieved through a variety of methodological approaches, as has occurred for other aspects of intestinal physiology.

Second, we wanted to replicate the finding of substantial apparent passive absorption of hydrophilic solute by house sparrows (Passer domesticus) (Caviedes-Vidal and Karasov, 1996) using probes other than radiolabeled 2-glucose. With this objective in mind, we used two carbohydrate probes similar in molecular size to 2-glucose (relative molecular mass, \( M_r = 180.2 \)), \( \text{D-mannitol} \) (\( M_r = 182.2 \); \( \text{C}_6\text{H}_{14}\text{O}_6 \)) and \( \text{L-arabinose} \) (\( M_r = 150.13 \); \( \text{C}_5\text{H}_{10}\text{O}_5 \); the natural isomer is dextro), which we analyzed by gas chromatography. On the basis of other studies cited below, we would expect the L- and D-isomers of both probes to be absorbed passively, as is thought to be the case for 2-glucose (see above). These studies pave the way for future studies using hydrophilic probes of different sizes that can be used to determine how passive absorption varies with molecular size.

Finally, as a first test of our new procedures with the new probes, we measured absorption from solutions with probe concentrations ranging from 1 to 1000 mmol l\(^{-1}\). When absorption rates increase linearly with substrate concentration, the kinetics is defined as first-order and the transport is considered to be passive and not mediated by a specific transport protein (such as the \( \text{Na}^+ \)-dependent glucose transporter, SGLT1), which would yield instead an absorption rate that increases sublinearly to a maximum. The absorption of both \( \text{D-mannitol} \) and \( \text{L-arabinose} \) is linear with concentration, i.e. non-mediated, in both humans and laboratory rats (Dawson et al., 1987; Kruglik et al., 1994), so we hypothesized that these probes are absorbed passively and therefore predicted that their absorption by house sparrows would be linearly related to solute concentration. Because our measure of absorption is influenced both by the instantaneous absorption rate at the intestinal apical membrane and by the contact time with the digesta (Lenner, 1995), we coupled our absorption measures with measures of digesta retention time made using the non-absorbable marker ferrocyanide (McWilliams and Karasov, 1998; Pappenheimer and Reiss, 1987; Sadowski and Meddings, 1993). If absorption rate at the intestinal surface is directly proportional to substrate concentration, and the contact time of digesta with the intestinal surface is independent of concentration, then we also predicted that the fractional absorption of these carbohydrate probes should be independent of probe concentration and should be substantial, like the apparent absorption of 2-glucose by the house sparrow. We use the house sparrow because its cosmopolitan distribution and ease of capture and laboratory maintenance make it a good subject for a variety of laboratory studies of avian physiology.

**Materials and methods**

**Birds and their maintenance**

House sparrows (Passer domesticus) were captured using mist nets in the vicinity of the Universidad Nacional de San Luis Campus, San Luis, Argentina. The birds were housed individually in cages (0.40 m x 0.40 m x 0.40 m) indoors under relatively constant environmental conditions (25.2±0.3°C, relative humidity 50±9%) on a photoperiod of 14 h:10 h L:D with ad libitum water and food (a mixture of seeds, vitamins and minerals; Gausch SA, Bahia Blanca, Argentina). Animals were acclimated to laboratory conditions for at least 15 days prior to use in experiments. The routine animal care and experimental procedures used in this study were reviewed and approved by the University of Wisconsin Research Animal Resources Center.

**Pharmacokinetic measurement of absorption**

To measure absorption, we used a pharmacokinetic technique that relies on the appearance of probes in the blood and involves feeding and injecting probes and then sampling the blood or excreta at various times post-gavage or post-injection (Caviedes-Vidal and Karasov, 1996). Birds were placed in special observation cages (Afik and Karasov, 1995). Oral gavage was performed using a cannula with a blunt edge inserted deeply through the esophagus into the stomach. Probes were always administered after 2 h of fasting, because we did not want to introduce the cannula into a stomach packed with food, and our data on retention time (see below) indicated that approximately two-thirds of water-soluble material clears in that time interval. The pulse consisted of the probes diluted in 500 \( \mu \)l of distilled water. The composition of the solution for each experiment is given in the Results section. Gavages were completed within 30 s. Injections were 50 \( \mu \)l of solution (100 mmol l\(^{-1}\) \( \text{mannitol} \) and/or 100 mmol l\(^{-1}\) \( \text{arabinose} \)) into the pectoralis. Before gavage or injection, we always checked the osmolalities of the solution using a vapor pressure osmometer (Wescor VAPRO 5520).

Blood samples (50 \( \mu \)l) were collected from the brachial vein into a heparinized capillary tube. The sample was centrifuged at 4000 g, and the plasma was separated and stored at 0°C for analysis. Feces were collected on cellophane paper lying on the bottom of the observation cages, carefully poured into Kahn tubes, diluted with distilled water (20:1; w/w) and processed immediately to avoid any decomposition of the substances to be determined.
Plasma proteins were precipitated using 10% ZnSO₄ and acetonitrile (Merck, Germany; HPLC quality) (Lam and Malikin, 1989). We gently agitated the tube for a few seconds and centrifuged at 4000 g for 3 min. The pellet was discarded, and the supernatant was vacuum-dried. With the dried sample we performed a syrillation reaction, which makes the compound volatile for gas chromatography, using 98% chlorotrimethylsilane (Aldrich, Milwaukee, USA) and hexamethyldisilazane (Sigma Chemical Co., St Louis, USA) in pyridine (Merck, Germany). The syrillation was performed 40 min before injection into the chromatograph.

The samples were injected into a gas chromatograph (Hewlett-Packard, model 5890, Series II plus) with an HP-5 column (5% phenyl-methyl-silicone) 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 concentrations, we integrated three peaks, at 7.8, 8.4 and 9.1 min for d-mannitol, we used the peak at 14.45 min. The software integrates the peaks and gives values in arbitrary units. To determine the concentrations of the unknown samples, we prepared calibration curves for each probe day every.

Diluted fecal sample was homogenized for 5 s at maximum speed with a tissue homogenizer (PowerGen 125) and centrifuged at 4000 g for 20 min. A sample of the supernatant was taken, and lipids were extracted using petroleum ether. Proteins were then precipitated, and the syrillation was performed before the injection into the chromatograph, as for blood samples.

Test for metabolism of probes and measurement of digesta retention time

Solutions containing the non-absorbable marker potassium ferrocyanide (10 mmol l⁻¹) and L-arabinose and/or D-mannitol (at concentrations specified in the Results section) were administered by oral gavage, and excreta were collected for 48 h. We collected excreta every time the bird defecated during the first 6 h after gavage, every hour until 12 h and then every 6 h until the 48 h had been completed. Carbohydrate probes in the excreta were analyzed as described above. Potassium ferrocyanide was assayed using a colorimetric method (Berliner et al., 1955). A 100 µl portion of the excreta sample was diluted with 900 µl of distilled water, 40 µl of 37% HCl and 40 µl of 10% hydrogen peroxide. The absorption of the mixture at 420 nm was read after 15 min. Each sample was assayed in duplicate. The calibration curve was prepared using 'clean' feces from the same birds dried at 60°C. The retention time was calculated as the sum of the products of the excretion time and the proportion of marker excreted during each time interval (Warner, 1981).

Data and statistical analyses

The absorption of carbohydrate probe was calculated by assuming a single compartment and first-order kinetics (Welling, 1986). We concluded that this assumption was appropriate on the basis of an inspection of plasma decay curves following injection of probes and by comparative fitting of the data to this model compared with a two-compartment model (see below). The excretion kinetics of mannitol in humans approximates a first-order, single-compartment system (Elia et al., 1987). In this instance, the amount of probe absorbed, A (in mg), is calculated as $A = \text{AUC} \cdot k_e \cdot S$ (equation 11.26 in Welling, 1986), where $\text{AUC}$ (the area under the curve; in mg mg⁻¹ plasma) is the area under the probe plasma concentration versus time curve, $k_e$ (in min⁻¹) is the elimination constant for removal of the probe from plasma, and $S$ (g plasma) is the distribution space of the probe in the plasma. The latter two variables were determined by injecting the probes, sampling plasma over time and fitting (non-linear curve-fitting; Gauss–Newton method; SYSTAT; Wilkinson, 1992) the plasma concentrations C (mg probe g⁻¹ plasma) at each time t to the equation $C(t) = C_0 e^{-k_e t}$. $C_0$, the intercept, is the dose (mg) injected divided by S. The value of the variable $\text{AUC}$ was determined on the basis of plasma probe concentrations at various times following oral gavage. Following typical procedures in pharmacokinetics (Welling, 1986), the area from $t=0$ to $t=\infty$ (min; when the final blood sample was taken) was calculated using the trapezoidal rule. The area from $t=0$ to $t=\infty$ was calculated as $\text{AUC} = \int_{0}^{\infty} C(t) dt$. The total $\text{AUC} \cdot k_e$ was obtained by summing the two areas. The fraction of probe absorbed, F, was calculated as $F = \text{AUC} / D$, where D is the dose (mg probe) administered by oral gavage and A is the amount of probe absorbed.

Numerical results are given as means ± S.E.M. (N is the number of animals unless indicated otherwise). Non-linear curve-fitting (Gauss–Newton algorithm, SYSTAT; Wilkinson, 1992) was used to fit kinetic data. Kinetic models were compared according to Motulsky and Ransnas (Motulsky and Ransnas, 1987). Least-squares regression was used to evaluate amounts absorbed versus concentration administered. For comparisons of single measurements made within individuals, repeated-measures analysis of variance (ANOVA) was performed. The F-values of these and other analyses of variance are presented in the text with the relevant degrees of freedom as subscripts.

Results

Absorption and elimination kinetics of carbohydrate probes

Elimination kinetics of mannitol were determined in the birds both prior to and following the absorption experiments. The mean body mass of the birds did not differ at these sampling times (respectively, 23.4±0.6 g before compared with 24.4±0.6 g after, N=6, F₁,₅=1.3, P=0.3). In the second elimination experiment, arabinose was injected simultaneously with mannitol (each at 100 mmol l⁻¹). The semi-logarithmic plots of plasma probe concentration following injection were linear (Fig. 1), implying single-compartment, first-order kinetics. This may reflect that the probes are cleared from the body by the kidney at a rate proportional to their concentration in the plasma. A two-compartment model did not significantly
Fig. 1. Plasma probe concentration as a function of time since injection of the probe. The plasma probe concentration is calculated as the measured concentration (µg probe g⁻¹ plasma) normalized to the amount of probe (µg) injected. This method of calculation and expression (the actual units are g⁻¹ plasma) allows for simple determination of the dilution space (g) for the probe, which is the inverse of the intercept (see Materials and methods). Data are shown for the same six house sparrows injected with mannitol (open circles, dotted line) prior to the absorption experiments and following the absorption experiments with both mannitol (filled circles, dashed line) and arabinose (filled triangles, solid line). The lines are least-squares fits that are included to help distinguish the data sets (see text for statistical comparison of the data sets).

Fig. 2. Plasma mannitol concentration as a function of time since feeding the probe. Each symbol represents a different individual (N=6 birds), and the lines connect the data points for each individual to help distinguish the data sets.

guided our subsequent measurements of probe absorption in three ways.

First, these data indicated that the time of the last blood sampling should vary according to gavage probe concentration: 30 min for 1 and 5 mmol l⁻¹ samples, 60 min for 10 and 25 mmol l⁻¹, and 80 min for higher concentrations. Second, the log-linear slope indicated that the rate constants for absorption (kₐ) and elimination (kₑ) were not similar to each other because in that case the relationship in Fig. 2 would have been a continuous convex curve (Welling, 1986, p. 171). Theoretically, the terminal log-linear slope equals either kₐ or kₑ, depending on which is larger (Welling, 1986), but in other experiments (below) we found that, within birds, the terminal slopes in the absorption experiments were the same as the values for kₑ in injection/elimination experiments. Thus, for each bird in each absorption experiment, we could use the terminal slope as an estimate of kₑ during that experiment, and we used this value in conjunction with the final blood sample to calculate \( AUC_{x}^{\infty} = (C \times t)/kₑ \) (see Materials and methods). Finally, because 93±1% of the total \( AUC_{x}^{\infty} \) could be estimated by sampling blood first at 10 min post-gavage, we omitted the earlier sampling point in subsequent absorption experiments to minimize stress on these small birds.

**Effects of probe concentration on probe absorption**

Absorption as a function of probe concentration was determined in two experimental sets. In the first set, using the same six individuals represented in Fig. 1, mannitol and arabinose were administered by gavage together at three concentrations in distilled water so that the total solute concentrations was 200 mmol l⁻¹ (i.e. 10 mmol l⁻¹ mannitol + 190 mmol l⁻¹ arabinose, or 100 mmol l⁻¹ of each or 190 mmol l⁻¹ mannitol + 10 mmol l⁻¹ arabinose). The terminal...
Fig. 3. Mean (± S.E.M.) plasma probe concentrations as a function of time since feeding the probes to six house sparrows. Mannitol is represented by filled symbols and arabinose by open symbols. Concentrations were 10 mmol l⁻¹ (circles), 100 mmol l⁻¹ (squares) and 190 mmol l⁻¹ (triangles). The lines are least-squares fits and are included to help distinguish the data sets (see text for statistical comparison of the data sets).

The slopes of the absorption curves were independent of concentration and not significantly different from those of the injection/elimination experiments for both mannitol (repeated-measures ANOVA; F₃,₁₅=1.22, P=0.34) and arabinose (F₃,₁₅=2.71, P=0.082) (Fig. 3). The calculated amount absorbed increased linearly with concentration (P<0.0001 for both solutes) and the least-squares fitted lines had intercepts not significantly different from zero for both arabinose (t₁₀=0.31, P=0.8) and mannitol (t₁₀=0.02, P>0.9) (Fig. 4). The slope of amount absorbed versus concentration for arabinose did not differ significantly from that for mannitol (repeated-measures ANOVA; F₁,₅=3.58, P=0.12). The fractional absorptions also did not differ significantly between the two probes or among the three concentrations (F₅,₂₅=1.7, P=0.18) and averaged 0.69±0.03 (N=6 birds).

In the second experimental set, we administered mannitol alone over a wider concentration range (1, 5, 10, 25, 200 and 1000 mmol l⁻¹) to nine other individuals, although not the same concentrations to every individual. In six of the individuals measured over the range 10–1000 mmol l⁻¹, the fractional absorptions did not differ significantly by concentration (repeated-measures ANOVA; F₂,₁₀=1.240, P=0.330), a result like that in the first experimental set. For all nine individuals, the calculated amount absorbed increased linearly with concentration (t₂=190, P<0.0001) and the intercept was not significantly different from zero (t₃₂=0.96, P=0.35) (Fig. 5). The slope for this experimental set (0.035±0.002; Fig. 5) was lower than that for mannitol in the first experimental set (0.054±0.005; Fig. 4), implying that less mannitol was absorbed, but the difference in fractional absorption was not significant (respectively, 0.60±0.04 versus 0.68±0.08, t₁₃=1.1, P=0.3). Fractional absorption of mannitol for all 15 individuals in experimental sets 1 and 2 (0.63±0.04) did not differ significantly from that of arabinose for the six individuals in experimental set 1 (0.69±0.06; t₁₉=0.88, P=0.39).

Fig. 4. Amounts of probe absorbed as a function of probe concentration fed to six house sparrows. L-Arabinose is represented by open triangles and D-mannitol is represented by open circles. The lines are the least-squares fit of the data for each probe. For arabinose, y=(−0.30±0.95)+(0.075±0.008)x (P<0.001 for the slope, P=0.7 for the intercept). For mannitol, y=(0.12±0.76)+(0.054±0.005)x (P<0.001 for the slope, P=0.9 for the intercept; means ± S.E.M.).

Fig. 5. The amount of mannitol absorbed as a function of mannitol concentration fed to nine house sparrows. The line is the least-squares fit: y=(0.77±0.79)+(0.035±0.002)x (P<0.001 for the slope, P=0.35 for the intercept; means ± S.E.M.).
Table 1. Mouth-to-cloaca retention time and recovery of the non-absorbable marker ferrocyanide administered orally to house sparrows

<table>
<thead>
<tr>
<th>Gavage solution (mmol l(^{-1}))</th>
<th>Solution osmolality (mosmol kg(^{-1}))</th>
<th>Retention time (min)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 potassium ferrocyanide + 10 D-mannitol + 100 l-arabinose</td>
<td>240±1</td>
<td>108±6</td>
<td>81±2</td>
</tr>
<tr>
<td>10 potassium ferrocyanide + 100 D-mannitol + 100 l-arabinose</td>
<td>247±1</td>
<td>112±5</td>
<td>81±1</td>
</tr>
<tr>
<td>10 potassium ferrocyanide + 190 D-mannitol + 10 l-arabinose</td>
<td>243±1</td>
<td>113±4</td>
<td>82±2</td>
</tr>
<tr>
<td>10 potassium ferrocyanide + 10 D-mannitol</td>
<td>43±2</td>
<td>113±5</td>
<td>83±2</td>
</tr>
<tr>
<td>10 potassium ferrocyanide + 200 D-mannitol</td>
<td>246±1</td>
<td>113±3</td>
<td>82±1</td>
</tr>
<tr>
<td>10 potassium ferrocyanide + 1000 D-mannitol</td>
<td>1024±2</td>
<td>114±4</td>
<td>82±2</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. for the same six individuals.

Digesta retention time

Because our measure of absorption is influenced both by the instantaneous absorption rate at the intestinal apical membrane and by the contact time with digesta, we measured digesta retention time using the non-absorbable marker ferrocyanide in the two sets of absorption experiments. Retention time did not vary significantly among experiments in which the carbohydrate probe concentrations and total solution osmolalities differed (repeated-measures ANOVA; \(F_{5,20}=0.3\), \(P=0.9\), Table 1). Recovery of the marker averaged 82% and also did not vary significantly among these treatments (repeated-measures ANOVA; \(F_{5,20}=1.9\), \(P=0.14\), Table 1).

Recovery of carbohydrate probes

After gavaging a solution containing 100 mmol l\(^{-1}\) mannitol and 100 mmol l\(^{-1}\) arabinose, we recovered in the excreta slightly more arabinose (77.1±2.4%) than mannitol (70.8±2.3%) (N=6, repeated-measures ANOVA; \(F_{1,5}=12.8\), \(P=0.016\)). Although we collected excreta for 24 h, most was recovered by 12 h (results not shown). When mannitol was injected, we recovered significantly more (83.7±1.1%) than when it was administered by gavage (N=6, repeated-measures ANOVA; \(F_{1,5}=35.5\), \(P=0.002\)). Recovery of injected mannitol also mainly occurred by 12 h post-administration (results not shown).

Discussion

The technique that we used to measure absorption of carbohydrate probes is widely used by pharmacokineticians to determine the absorption (bioavailability) of drugs and toxins. This approach is required when working with non-mammalian vertebrates in which urinary wastes are pooled with fecal wastes. A simpler approach in mammals is to feed the probes and recover them in the urine, and this is the basis for clinical tests and scientific studies of intestinal permeability (Elia et al., 1987; He et al., 1996; Maxton et al., 1986). These pharmacokinetic methods have multiple advantages over perfusion studies with anesthetized animals in that they are simple, avoid possible artifacts of anesthesia (Ubing and Kimura, 1995a) and replicate the natural situation; indeed, we have also used them on feeding animals (Caviedes-Vidal and Karasov, 1996).

Ideal intestinal permeability probes are (i) inert, (ii) non-absorbable through mediated transport, (iii) normally not present in the organism and (iv) non-metabolizable (Hamilton et al., 1987). In our case, we found that the markers used meet most of the above requirements. They were administered without apparent harm to house sparrows at concentrations up to 1000 mmol l\(^{-1}\). To our knowledge, there is no evidence of mediated intestinal transport of either arabinose or mannitol (Dawson et al., 1987; Krugliak et al., 1994), and our results strongly suggest that the absorption of these molecules was passive (below). These probes are not normally present in sparrows. We recovered most of the probes following oral or intramuscular injection, the same proportion, in fact, as of the putative non-absorbable marker ferrocyanide. The inability to collect and/or measure absolutely all probe excreted accounts for some of the missing probe, but small amounts might be metabolized by organs such as the liver (Wick et al., 1954) or by microbes in the gut, as implied by the 12% difference in the comparison of unrecovered ingested with injected mannitol. Because our absorption calculations rely on amounts in blood relative to administered doses, our absorption estimates and conclusions should be little affected by the small losses.

Our estimate of substantial absorption of mannitol and arabinose in direct correlation with their orally administered concentrations is robust. We assumed that absorption and elimination kinetics were single-pool and first-order. Our evaluation of post-injection plasma levels indicated a log-linear relationship in support of this assumption. Further, the terminal portions of the absorption curves (Figs 2, 3) were log-linear and parallel for all solutions administered, and even parallel with the line for elimination (c.f. Figs 1, 3), indicating a probe elimination rate constant independent of plasma probe concentration. The probe distribution spaces of mannitol (5.6 g) and arabinose (8.8 g) were similar to that for L-glucose (6.4 g; Caviedes-Vidal and Karasov, 1996), and all correspond approximately with the extracellular fluid space of a vertebrate (25% of body mass; Schmidt-Nielsen, 1990), although there is no strict requirement in pharmacokinetics that a distribution space correspond with any physical entity. Our finding of a larger distribution space for arabinose than for mannitol is not critical, but it is curious because both are, for the most part,
Absorption of hydrophilic probes by house sparrows

water-soluble, and we have no simple explanation for it except that perhaps it relates to the slightly higher water solubility of mannitol than arabinose \[ \log(\text{octanol-water partition coefficients}), \] respectively, \(-4.67\) and \(-2.86\); Prusnitz and Noonan, 1999). As an alternative to assuming single-pool first-order kinetics, one could use the standard model-independent method for calculating fractional absorption in which \( AUC_{\text{norm}} \) from an absorption experiment is divided by \( AUC_{\text{norm}} \) from an injection/elimination experiment (Welling, 1986). Considering the similar shapes of our absorption and elimination curves and the fact that we made our final plasma measurements at levels near the resolution limit of the gas chromatograph/mass spectrometry system, our results with this method would have been similar.

At first consideration, it seems counter-intuitive that, when similar molal concentrations of arabinose and mannitol were administered, nearly twice as much mannitol appeared in the blood (Fig. 3), especially because passive absorption through a sieve might be considered proportional, in part, to molecular size (Hamilton et al., 1987; He et al., 1996) and arabinose is smaller than mannitol. However, because mannitol has a higher molecular mass than arabinose, oral gavage with 500 \( \mu \)l of 100 mmol l\(^{-1} \) of the former delivers more mass (9.11 mg) than of the latter (7.51 mg) to be absorbed into plasma. Second, the injection/elimination experiments shown in Fig. 1 indicated that the dilution space of arabinose (8.8 g) was larger than that for mannitol (5.6 g), so that whatever arabinose gets absorbed is diluted more than whatever mannitol is absorbed, and this further reduces the plasma concentration (\( \mu \)g g\(^{-1}\) plasma). The total increase for mannitol, compared with arabinose, is 1.21 for the molecular mass difference (\( \approx 911/751 \)) and 1.57 for the dilution space difference (\( \approx 8.8/5.6 \)), for a total increase of 1.90-fold in initial plasma concentration (\( \mu \)g g\(^{-1}\) plasma), assuming the same fractional absorption. Although arabinose might be expected to have a higher fractional absorption because of its smaller molecular mass, the difference we observed between arabinose and mannitol (respectively, 0.69 versus 0.63) was not significant. However, preliminary data in other experiments with house sparrows indicate that the fractional absorption of carbohydrate probes (arabinose, rhamnose, persitol, lactulose) declines with increasing molecular mass (J. G. Chediack, E. Caviedes-Vidal and W. H. Karasov, unpublished data).

Our use of fractional absorptions of orally administered probes to make inferences about absorption kinetics rests on the logical argument that the fractional absorptions are positively related to rates of absorption at the intestinal brush border (Lennermans, 1995). This would not be true if the fractional absorption were 1.0, because then even longer digesta retention times would not result in higher fractional absorptions. However, our fractional absorptions were less than 1.0 (average 0.69). In fact, retention times were constant in our experiments (Table 1), and we therefore conclude that the linear variation in amount of carbohydrate probe absorbed with amount administered (Figs 4, 5) probably reflects a linear relationship between luminal concentration and absorption rate, the hallmark of a passive process. Researchers using perfusion experimental models and \( D \)-mannitol as a marker reported the same relationship (Gato-Peciña and Ponz, 1990; Krugliak et al., 1994). Furthermore, our approach is consistent with theoretical modeling of drug absorption that predicts, in the presence of a saturable process, a declining fractional absorption with increased substrate concentration, which we did not observe, and dose-independent fractional absorption in the presence of solely passive absorption (Yu and Amidon, 1998), which we did observe. Measurements of fractional drug absorption have supported the theoretical model predictions (Matsuda et al., 1998; Yu and Amidon, 1998).

The exact probe concentrations adjacent to the intestinal surface were unknown, and for some solutions were probably lower than the concentration that was delivered by oral gavage. For example, the solution containing 1000 mmol l\(^{-1} \) mannitol in the second experimental set (see Results) was hyperosmotic in relation to blood and could have caused osmotic water flux into the lumen, diluting the probe. However, in the first experimental set, the solutions were slightly hypo-osmotic (200 mmol l\(^{-1} \)) and the osmolality was constant over all concentrations of mannitol and arabinose, so we suspect that probe concentrations at the intestinal surface were at least proportional to concentrations in the gavage solutions, if not approximately the same. The difference in solution osmolality might account for the slightly lower slope for mannitol shown in Fig. 5 compared with Fig. 4. An alternative way to display the plots in Figs 4 and 5 would be to plot absorption as a function of mass or \( \mu \)moles of probe gavaged, but our major conclusion of substantial probe absorption would hold nonetheless.

Our results are consistent with our earlier report (Caviedes-Vidal and Karasov, 1996) of substantial (75%) absorption by intact house sparrows of \( L-H \)glucose, the stereoisomer that does not interact with the \( D \)-glucose transporter (SGLT1). The confirmation of a substantial pathway for passive absorption was important because of concerns that results based on \( L-H \)glucose could be confounded by changes in radiopurity (Caviedes-Vidal and Karasov, 1996) or interactions with SGLT1 (Schwartz et al., 1995). Our results are also consistent with a very early report of extensive absorption of arabinose by humans (56%) and intact rats (31%) (McCance and Mudders, 1930) that, remarkably, presaged modern pharmacokinetic approaches such as ours.

Because transcellular diffusion across the apical and basolateral membranes of epithelial cells is extremely low for hydrophilic compounds, we assume that most of the absorbed mannitol and arabinose traversed through the paracellular junctional areas between enterocytes. A number of investigators have concluded that mannitol absorption is paracellular on the basis of autoradiography or the observed effects on absorption of specific substances that vary the structure and, hence, the permeability of the intercellular junctions (Gato-Peciña and Ponz, 1990; Ma et al., 1993; Krugliak et al., 1994). Our results shed no light on whether the flux is diffusive or convective (i.e. probe dragged passively with solvent flux). Furthermore, we cannot rule out other
putative non-selective mechanisms, such as a process involving mucosal endocytosis and serosal exocytosis, which we think is very unlikely considering the high fractional absorption we observed.

The very idea of a substantial non-selective, passive pathway in animals seems implausible to some (Diamond, 1991) because one might predict natural selection against a pathway that could expose animals to hydrophilic toxins in their foods. However, our results with l-glucose, arabinose and mannitol add to an accumulating body of evidence that, in some vertebrates, small to medium-sized hydrophilic solutes such as creatinine (Pappenheimer, 1990), 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) are absorbed largely via a paracellular route. The occurrence and possible regulation of this route (Anderson and Van Itallie, 1995; Ballard et al., 1995) have important implications for nutrition and drug design. Furthermore, vulnerability to hydrophilic toxins could be an important ecological driving force, constraining food exploratory behavior, limiting the breadth of the dietary niche and selecting for compensatory behaviors such as searching for and ingesting specific substances that inhibit hydrophilic toxin absorption (Diamond et al., 1999). Our method for measuring probe absorption following single oral administration should facilitate the study of these topics.

We thank Lorena Carvelli, Ana Brigada and the Membrane Group of CREACYT for assistance in many phases of this study. This research was supported by FONCYT (01-03101) and UNSL grant 9502 to E.C.V. Support to W.H.K. came from National Science Foundation Grant IBN-9723793, the A.W. Schorger Fund and the Max McGraw Wildlife Foundation.

References


Maxton, D. G., Bjarnason, I., Reynolds, A. P., Catt, S. D., Peters,


