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L-glucose absorption in house sparrows (*Passer domesticus*) is nonmediated

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Abstract We previously demonstrated in intact house sparrows substantial absorption in vivo of L-glucose, the stereoisomer of D-glucose that is assumed not to interact with the intestine's D-glucose transporter. Results of some studies challenge this assumption for other species. Therefore, we tested it in vitro and in vivo, based on the principle that if absorption of a compound (L-glucose) is mediated, then absorption of its tracer will be competitively inhibited by high concentrations of either the compound itself or other compounds (e.g., D-glucose) whose absorption is mediated by the same mechanism. An alternative hypothesis that L-glucose absorption is primarily paracellular predicts that its absorption in vivo will be increased (not decreased) in the presence of D-glucose, because the permeability of this pathway is supposedly enhanced when Na^+-coupled glucose absorption occurs. First, using intact tissue in vitro, we found that uptake of tracer-radiolabeled L-glucose was not significantly inhibited by high concentrations (100 mM) of either L-glucose or 3-O-methyl-D-glucose, a non-metabolizable but actively transported D-glucose analogue. Second, using intact house sparrows, we found that fractional absorption of the L-glucose tracer was significantly increased, not reduced, when gavaged along with 200 mM 3-O-methyl-D-glucose. This result was confirmed in another experiment where L-glucose fractional absorption was significantly higher in the presence vs. absence of food in the gut. The greater absorption was apparently not due simply to longer retention time of digesta, because no significant difference was found among retention times. Our results are consistent with the idea that L-glucose is absorbed in a non-mediated fashion, largely via the paracellular pathway in vivo.

Keywords Intestinal absorption · Passive transport · Pharmacokinetic · Bioavailability · Absorption rate

Abbreviations *AUC* area under the curve · *3OMD-glucose* 3-O-methyl-D-glucose

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Introduction

L-glucose, the stereoisomer of D-glucose, has a variety of uses in physiology including as a probe to study extracellular glucose distribution (Steil et al. 1996), and to study passive absorption of D-glucose in particular (Karasov and Diamond 1983; Uhing and Kimura 1995), or passive absorption of hydrophilic compounds generally (Yuasa et al. 1995). Its use is often based on the assumption that L-glucose does not interact with the D-glucose or other transporter(s), an assumption supported by the observation that uptake of tracer L-glucose is not influenced by unlabeled L- or D-glucose in bulk solution, at least in some mammals tested (Thomson et al. 1982; Meddings and Westergaard 1989). However, results of some other studies challenge this assumption. Early work with hamster everted sacs indicated that L-glucose accumulation into sacs was inhibited by D-glucose and phlorizin (Caspary and Crane 1968). More recently, Schwartz et al. (1995) found that much more L-glucose than other similar-sized sugars (L-mannose, D-mannitol) was absorbed from drinking solutions by laboratory rats, and that co-administration of D-glucose or food significantly reduced L-glucose absorption, as if its absorption were competitively inhibited. Because we previously found that 75–90% of ingested L-glucose was absorbed

and excreted in urine of a number of species of intact birds (Karasov and Cork 1994; Caviedes-Vidal and Karasov 1996; Levey and Cipollini 1996; Afik et al. 1997), we decided to test the assumption of passive L-glucose absorption using one of the species, house sparrows (*Passer domesticus*).

Our tests were based on the principle that if absorption of a compound (L-glucose) is mediated, then absorption of its tracer, radiolabeled L-glucose, will be competitively inhibited by high concentrations of either the compound itself or other compounds (e.g., D-glucose) whose absorption is mediated by the same mechanism (Malo and Berteloot 1991). An alternative hypothesis that L-glucose absorption is primarily paracellular predicts that its absorption in vivo will be increased (not decreased) in the presence of D-glucose. This is based on findings in mammals that hydrophilic probes cross the intestinal mucosa by diffusion and/or solvent drag via a paracellular pathway, and that the permeability of this pathway is enhanced when Na^+-coupled nutrient transport occurs (Madara and Pappenheimer 1987; Pappenheimer 1987; Pappenheimer and Reiss 1987; Pappenheimer and Volpp 1992; Sadowski and Meddings 1993; See and Bass 1993; Turner and Madara 1995). The Na^+-coupled transport is thought to alter the cellular cytoskeleton, resulting in rearrangement of tight-junction complexes thereby increasing paracellular permeability to water and hydrophilic molecules, and/or to increase paracellular water flux and solvent drag.

To test for mediated absorption of L-glucose, we measured its absorption in intact tissue in vitro and in intact animals in the presence and absence of high concentrations of L- and D-glucose and other nutrients. For in vivo measurement we applied standard pharmacokinetic methods to measure the extent of L-glucose absorption. Extent, or fractional, absorption is influenced by the instantaneous absorption rate at the intestinal apical membrane as well as the contact time with digesta (Lennernas 1995). Therefore, in one experiment we coupled our absorption measures with measures of digesta retention time made using the nonabsorbable marker ferrocyanide (Pappenheimer and Reiss 1987; Sadowski and Meddings 1993; McWilliams and Karasov 1998). If absorption rate at the intestinal surface is faster in the presence of luminal nutrients, and the contact time of digesta with the intestinal surface is not altered, then the fractional absorption should be higher.

Materials and methods

Birds and their maintenance

House sparrows (*P. domesticus*) were captured in the vicinity of the campuses of University of Wisconsin–Madison, U.S.A. (for Experiment 1 and Experiment 2) or Universidad Nacional de San Luis, Argentina (for Experiment 3). They were housed in cages (0.80 m×0.60 m×0.60 m) indoors under relatively constant environmental conditions (25.0 ± 0.5 °C, relatively humidity 50 ± 9%, photoperiod 12:12 light:dark) and received ad libitum water and commercial bird seed mix. Sparrows were acclimated to laboratory conditions for at least 2 weeks prior to use in experiments.

Experiment 1: measurement of L-glucose and D-glucose uptake in vitro

As a quantitative test of whether L-glucose uptake is passive, we tested whether uptake of tracer radiolabeled L-glucose was inhibited by high concentration of unlabeled L-glucose or 3-O-methyl-D-glucose (3OMD-glucose). 3OMD-glucose is a non-metabolizable analogue of D-glucose that competes for the same apical cotransporter(s) as D-glucose (Solberg and Diamond 1987), such as SGLT1 and GLUT2 (Kellett 2001), and has a similar maximal transport rate as D-glucose in mammals (Thomson et al. 1982). If L-glucose uptake were passive, then nonlabeled L-glucose concentration would have no significant effect on radiolabeled L-glucose uptake. Alternatively, if uptake were mediated then uptake of the radiolabeled L-glucose would decline with increasing nonlabeled L-glucose concentration because of competition between labeled and unlabeled compound for the transporter (Malo and Berteloot 1991). In our experiments we also measured radiolabeled 3OMD-glucose uptake in the presence and absence of unlabeled 3OMD-glucose as a positive control to confirm the bioactivity of the prepared everted intestine sleeve as well as to demonstrate the inhibition of uptake of a radiolabeled tracer whose uptake is known to be partly mediated.

Everted sleeves of intestine mounted on rods were used to measure uptake of [^{14}C]L-glucose and [^{3}H]3OM D-glucose (American Radiolabeled Chemicals St. Louis, Mo.) in avian Ringer solutions composed of (in mM): 161 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, and 20 $NaHCO_3$; gassed with 95% O_2–5% CO_2 to yield pH 7.3–7.4 at 40 °C, 350 mosmol (isosmotic with avian blood). Solutions containing glucose or mannitol were prepared by isosmotic replacement of NaCl. Our procedures followed closely those described in Karasov and Diamond (1983) and Caviedes-Vidal and Karasov (1996), with only a few modifications. Birds were killed with an overdose of IsoFlo (isoflurane, Abbott Laboratories) and the intestine removed and perfused with cold saline. From each of eight birds we prepared nine adjacent intestinal sleeves, which were divided into groups of three sleeves, and each of the three sleeves was randomly assigned to incubation solutions with either unlabeled 100 mM mannitol, L-glucose, or 3OMD-glucose. After incubation and blotting on a filter paper, tissues 1 cm in length were cut and removed from the rod, weighed, and disintegrations per minute (dpm) were determined by liquid scintillation (Tracor Analytic, MarkIII, USA). For both D- and L-stereoisomers the probe space (dpm tissue^{-1}/dpm μl^{-1} incubation solution) was calculated and normalized to both intestine length and mass. Thus, the prediction was that the L-glucose space in the tissue would be independent of treatment (because no component of the uptake is mediated) whereas the 3OMD-glucose space would be reduced in the presence of high concentration of nonlabeled 3OMD-glucose.

Some tissues were also visually examined for any obvious damage during the everted sleeve preparation (Starck et al. 2000). Three groups of everted sleeves were saved from the experiments described above including the tissue before eversion, after eversion, and after incubation. All the tissue samples were quickly transferred into Z-5 (Anatech) overnight followed by 70% ethanol incubation. Tissue samples were imbedded into paraffin following the standard ethanol dehydration procedure. Vertical cross sections of ~20–25 μm thick were collected from blocks randomly selected from each group using a microtome (Leica 2035, Germany) and subsequently examined by light microscopy.

Experiment 2 and Experiment 3: measurement of L-glucose absorption in vivo

The primary purpose of these experiments was to test whether L-glucose absorption is decreased or increased in the presence of luminal nutrients. Two experiments were carried out in

which luminal nutrients were increased in two ways, by gavage (Experiment 2) or by meal (Experiment 3). We applied a pharmacokinetic method focusing on appearance of probes, like radiolabeled or unlabeled L-glucose, in blood to measure absorption. This method involves feeding and injecting the same probes and sampling blood or excreta at various times post-feeding or post-injection (Welling 1986; Caviedes-Vidal and Karasov 1996; Chediack et al. 2001).

Experiment 2

Food was removed from birds overnight prior to the experiment. Measurements began 2.5–3 h after lights went on in the morning. For absorption experiments, two groups of seven and six house sparrows were gavaged with 500 µl of a solution containing, respectively, 200 mM mannitol or 200 mM 3OMD-glucose, 10 µCi L-[^{14}C] glucose (American Radiolabeled Chemicals St. Louis, Mo.), and 80 mM NaCl. Oral gavage was performed using a stainless steel cannula with a blunt edge inserted through the esophagus deeply into the stomach. Gavages were completed within 30 s. Elimination experiments were performed using the same individuals, which were injected with L-[^{14}C] glucose (5 µCi in 250 µl 200 mM mannitol and 80 mM NaCl) into the pectoralis on days at least 1 week from the days when the gavage experiments were performed. The order of gavage or injection experiments performed on the same individual was random. The total osmotic pressure of gavage or injection solutions was controlled at 360 ± 10 mosmol (Wescor Vapor Pressure Osmometer 5520), and aliquot samples were saved for radioactivity analysis to quantify how much tracer was given in the experiment. Following either gavage or injection, seven-to-nine blood samples (20 µl each, 160 µl in total, which accounts for less than 10% of blood from normal house sparrows; Stangel 1986) were collected from the brachial vein with heparinized capillary tubes over the subsequent 3–4 h. All the blood samples collected were analyzed immediately for radioactivity as described previously (Caviedes-Vidal and Karasov 1996). Briefly, blood was centrifuged (1,500 *g*) for 5 min, and plasma samples were weighed ($10–20 \pm 0.1$ mg) and then mixed with 2.0 ml scintillation fluor (Hionic-Fluor, Packard, USA) and counted in a scintillation spectrometer (Tracor Analytic, MarkIII, USA) for disintegration per minute (dpm) with automatic external standardization (AES) and background correction.

Three aliquot samples from radioactive solution were saved before it was gavaged into the sparrows and plasma samples from three different birds gavaged with radioactive solution were also collected for radiopurity testing. Radiopurity was checked by high performance liquid chromatography (HPLC) using an NH_3 column (Alltech, USA) and an acetonitrile:water = 85:15% mobile phase. We found that essentially all ^{14}C activity was associated with L-glucose in the solution ($96.7 \pm 2.6\%$) and in plasma ($92.6 \pm 2.5\%$).

Experiment 3

Six individuals were studied with either the gut empty or containing food. For the former, food was removed from birds overnight prior to the experiment, which has proved to produce an empty small intestine (E. Caviedes-Vidal, personal observation). Measurements began 2.5–3 h after lights went on in the morning. Five hundred microliters of distilled water containing 1 M L-glucose was gavaged within 30 s. For the elimination experiment, 150 µl of 2 M L-glucose was injected into the pectoralis of each bird. Blood samples of 75 µl were collected from the branchial vein with heparinized capillary tubes following either gavage or injection. Three to four blood samples (225 µl total, which accounts for less than 10% of the blood from normal house sparrows; Stangel 1986) were taken over the next 1.5 h. The samples were centrifuged at 4,000 rpm and the plasma was separated and stored at 0 °C for further analysis.

The concentration of L-glucose was determined indirectly by measuring the difference between total D- and L-glucose (anthrone method; Yemm and Willis 1954) and D-glucose by stereospecific method (enzymatic method, Glicemia enzimática. Wiener, Argentina). The anthrone method is based on the oxidation of anthrone to anthraquinone in the presence of soluble carbohydrates. An aliquot of 100 µl plasma diluted in distilled water 30:1 (w/w) was incubated for 10 min in an ice-cold water bath. Then, 1 ml reagent, containing 0.0918 g thiourea and 0.0918 g anthrone dissolved in 69.4 ml nitrogen-free sulfuric acid and 30.4 ml distilled water, was added slowly to prevent the solution temperature from increasing to above 25 °C. The tube containing the mix solution was then immediately placed in a boiling water bath for 20 min and the reaction was terminated by placing the tube in an ice-cold water bath. Thirty-minutes later, samples were read in a spectrophotometer (Milton Roy Spectronic model 21D) at 620 nm to yield total soluble carbohydrate concentration. Standard solutions were prepared over a range from 0.03125 g l^{-1} to 0.25 g l^{-1}.

A colorimetric enzymatic method was used to determine D-glucose concentration (Glicemia enzimática, Wiener). The method is based on glucose being first oxidized to gluconic acid and hydrogen peroxide, catalyzed by glucose oxidase (GOD). The hydrogen peroxide formed then reacts in the presence of peroxidase (POD), 4-aminofenazone, and phenol to form a quinone dye, which maximally absorbs at a wavelength of 505 nm. An aliquot of 10 µl diluted plasma sample was added to distilled water 2:1 (w/w) and 1 ml reagent, which contains 50 ml phenol reagent, 50 ml 4-aminofenazone, 900 ml distilled water, and 3 ml GOD/POD mix, and the solution was incubated for 10 min in water bath at 40 °C. Samples were read with a spectrophotometer at 505 nm. The concentration of L-glucose was determined by subtracting the D-glucose concentration from the total soluble carbohydrate concentration (Yemm and Willis 1954).

Measurement of digesta retention time

Digesta retention time was measured as described previously (Chediack et al. 2001). Briefly, solutions containing the non-absorbable marker potassium ferrocyanide (10 mM) and L-glucose (1 M) were administered by oral gavage, and excreta were collected for 6 h, during which time most marker is excreted (Chediack et al. 2001). Every time the bird defecated, excreta samples were collected on cellophane paper lying at the bottom of the observation cage. The potassium ferrocyanide concentrations were determined using a colorimetric method (Berliner et al. 1955). An aliquot of 100 µl excreta sample was diluted with 900 µl distilled water, 40 µl 37% HCl, and 40 µl 10% hydrogen peroxide. The mix was read 15 min later at 420 nm. Each sample was assayed in duplicate. The calibration curve was prepared using "clean" excreta of 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).

Pharmacokinetic calculations

The amount of L-glucose in each plasma sample at time t was normalized to the weight of each sample (C_t; µg L-glucose mg plasma^{-1} or dpm mg plasma^{-1}) and plotted against sampling time. The amounts of L-glucose absorbed were calculated from areas under the post-absorption and post-injection plasma curves (AUC; area under the curve of plasma L-glucose concentration vs. time: in units of dpm×min mg plasma^{-1} or mg L-glucose×min mg plasma^{-1}; Chediack et al. 2003). 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 $\text{AUC}^{x \to \infty} = C_t$ (at $t = x)/k_{\text{el}}$. The parameter k_{el} (min^{-1}) is the elimination constant for removal of the L-glucose from plasma, which was estimated by regressing (least squares regression;

SYSTAT, Wilkinson 1992) the last three log-transformed plasma concentrations C (mg probe g plasma^{-1}) against t and calculating the slope. The total $AUC^{0\rightarrow\infty}$ was obtained by summing the two areas ($AUC^{0\rightarrow x} + AUC^{x\rightarrow\infty}$).

Statistical analysis

Numerical data are presented as means ± SE (n = number of animals). Although data shown in the figures are sometimes pooled across individuals for the convenience of presentation, statistical analyses were performed based on data for individuals. Results were analyzed by repeated measures ANOVA and Student's t-test (SAS, SAS Institute, Cary, N.C.). The T- and F-values of these and other analyses of variance are presented in the text with the relevant degrees of freedom as subscripts. Linear regression analysis and multiple comparisons followed the method of least-square means and Tukey test. One-tailed tests were used for a priori predictions. Statistical significance was accepted for $P < 0.05$.

Results

Experiment 1: measurement of L-glucose and 3OMD-glucose uptake in vitro

The [^{14}C]L-glucose space did not differ significantly among tissues incubated in 100 mM of either mannitol, L-glucose, or 3OMD-glucose ($F_{2,32} = 1.06$, $P = 0.36$; Fig. 1). Within each treatment group, [^{14}C]L-glucose spaces were significantly lower (by an average of 37%) than those for [^{3}H]3OMD-glucose measured simultaneously in the same intestinal sleeves (Student's paired t-test, $P < 0.01$). Uptake of [^{3}H]3OMD-glucose was significantly inhibited by unlabeled 3OMD-glucose in the solution, but not by mannitol or L-glucose ($F_{2,32} = 5.76$, $P = 0.0073$, followed by pair-wise comparisons). Thus, uptake of 3OMD-glucose but not L-glucose was shown to be partly mediated on the basis of self-inhibition.

Histological evaluation of the tissues revealed no severe damage to the house sparrow small intestine (results not shown). As found previously with mouse and chicken intestine (Starck et al. 2000), after eversion, the villi appeared shorter and more squat but the tissue integrity and the cytology of the enterocytes were not distorted. The incubation did not appear to cause any further alteration.

Experiment 2: L-glucose absorption in vivo in the presence and absence of 3OMD-glucose

L-glucose in the circulatory system was cleared rapidly, with apparently ≥90% of elimination occurring over the course of a typical 4-h trial (Fig. 2). For the L-[^{14}C] glucose that was injected, the plasma radioactivity decreased log-linearly (mean r^2 value = 0.97 ± 0.01, $n = 13$ birds, all values of $P < 0.005$). The mean slope, or elimination rate constant, k_{el}, for the injection experiments (0.0215 ± 0.0011 min^{-1}; $n = 13$) did not differ significantly from the terminal slopes of the absorption curves for mannitol and 3OMD-glucose groups, which were, respectively, 0.0190 ± 0.0018 min^{-1} and

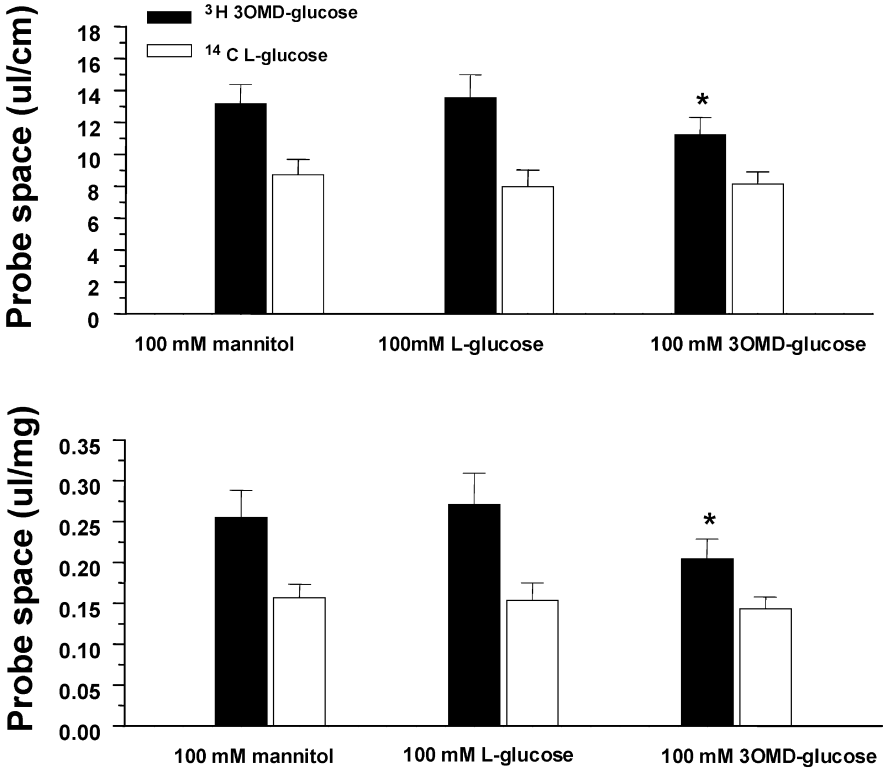

Fig. 1 Uptake of 3-O-methyl-D-glucose (*3OMD-glucose*), but not L-glucose was self-inhibitable and thus partly mediated. Everted small intestine sleeves of house sparrows were used to measure uptakes of [^{14}C] L-glucose and [^{3}H] 3OMD-glucose probe in the presence of either 100 mM unlabeled mannitol, L-glucose, or 3OMD-glucose. Uptakes (probe spaces; mean ± SE) are normalized to intestinal length (*upper graph*) and mass (*lower graph*). The *asterisk* denotes the significantly lower uptake of [^{3}H] 3OMD-glucose ($P = 0.0073$) in the presence of 100 mM unlabeled 3OMD-glucose compared to the other two treatment groups. The data validated our use of L-glucose as a probe for passive absorption and showed the bioactivity of everted intestine sleeves because D-glucose uptake (sum of active and passive uptake) significantly exceeded L-glucose uptake (passive only)

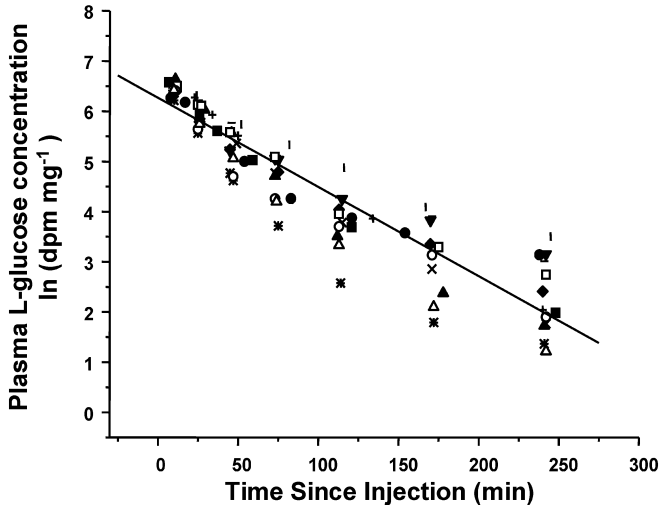

Fig. 2 Semi-log plot of plasma L-[^{14}C] glucose concentration as a function of time since injecting the probe to 13 house sparrows in Experiment 2. Seven to eight blood samples were taken from each bird over 4 h. Each individual is represented by a different *symbol*. The *line* shown is the least squares fit for all the data: $y = 6.269(\pm 0.045) - 0.0178(\pm 0.0004)x$

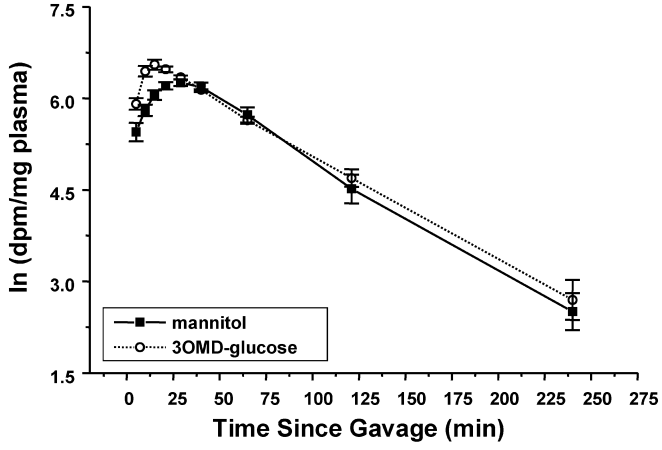

Fig. 3 Plasma L-[^{14}C] glucose concentration as a function of time since gavaging to nonfed house sparrows the probe with 200 mM mannitol ($n = 7$) or with 200 mM 3OMD-glucose ($n = 6$). For the purpose of comparison, the data collected for each group within 5% of each designated sampling time point were averaged ($\pm$SE), with the respective *lines* connecting the means. Note that the concentration of L-[^{14}C] glucose in the plasma peaked in the 3OMD-glucose groups earlier (at 15 min) than that for the mannitol group (at 30 min). Terminal slopes representing probe elimination for birds gavaged with 200 mM mannitol or with 200 mM 3OMD-glucose are the least squares fit for all the data in the respective groups: for mannitol: $y = 6.881(\pm 0.050) - 0.01843\ (\pm 0.0004)x$; for 3OMD-glucose: $y = 6.807(\pm 0.014) - 0.0172\ (\pm 0.0001)x$

0.0173 ± 0.0012 min^{-1} (Student's paired *t*-test, $T = 0.25$, $P = 0.81$, and $T = 0.97$, $P = 0.38$, respectively; Fig. 3).

When the birds were gavaged with L-[^{14}C] glucose, the concentration of this radiolabeled carbohydrate in the plasma peaked within 15 min for 3-O-MG treatment group, earlier than for the mannitol group (peak at 30 min) (Fig. 3). For each bird in each absorption experiment we used the terminal slope as an estimate of k_{el} during the experiment, and we used this value in conjunction with the final blood sample to calculate $AUC^{x \to \infty}$ and fractional absorption. The fractional absorption of radiolabeled L-glucose was significantly higher when administered to birds in solution with 200 mM 3OMD-glucose than when administered with 200 mM mannitol (respectively, 0.78 ± 0.02 vs.0.69 ± 0.03; Student's *t*-test, $T_9 = -2.67$, one-tailed $P = 0.013$).

Experiment 3: L-glucose absorption in vivo in the presence and absence of luminal nutrients

The patterns of absorption and clearance of L-glucose from plasma were similar to those in Experiment 2, and so the data are not shown. The fractional absorption of L-glucose in fed sparrows (0.51 ± 0.03; $n = 6$) was significantly higher than in sparrows not fed (0.43 ± 0.02; $n = 6$) (Fig. 4; Student's paired *t*-test, $T_5 = 2.33$, one-tailed $P = 0.034$).

There was no apparent difference in the amount of time over which absorption occurred, at least based on similar marker retention times in birds fed (75.7 ± 5.1 min; $n = 7$) and not fed (79.6 ± 6.1 min; $n = 5$) (repeated measures ANOVA, $F_{1,4} = 1.060$; $P > 0.3$).

Discussion

In the sections below we discuss how our findings are notable in two ways: first because they validate the use of L-glucose as a probe for passive absorption; second, because we found evidence of modulated passive absorption in the presence of luminal nutrients.

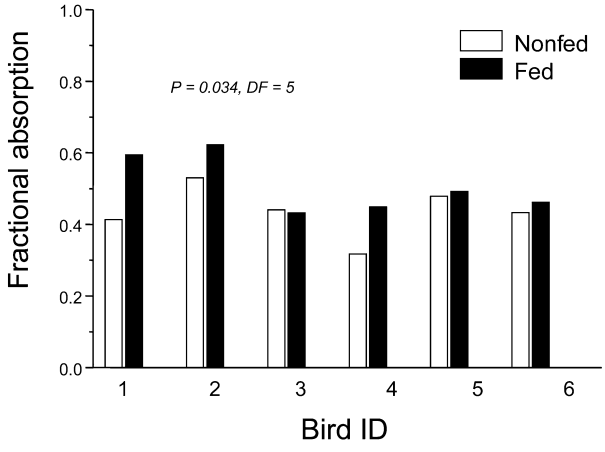

Fig. 4 The fractional absorption of L-glucose was significantly higher in house sparrows fed (*filled columns*) than nonfed (mainly overnight; *unfilled columns*) in Experiment 3 (one-tailed Student's paired *t*-test $T_5 = 2.33$, $P = 0.034$)

L-glucose as a probe for passive absorption

By measuring 3OMD- and L-glucose uptake in vitro, we demonstrated two major points. First, L-glucose uptake is most likely passive and non-mediated because, as expected for such a substrate, uptake of probe L-glucose was not influenced by unlabeled L- or 3OMD-glucose at high concentrations (100 mM; Fig. 1). Readers may be familiar with the more traditional method of testing for passive absorption by measuring total uptake over many substrate concentrations and then testing for linearity. That method is not inherently any more powerful than this method, which is based on competitive inhibition (Malo and Berteloot 1991), nor is it more physiological because it includes lower glucose concentrations more similar to those found in the lumen, which are probably in the range of 1–100 mM (Ferraris et al. 1990). In fact, the primary purpose is not to use "physiological" conditions but to use conditions most likely to elicit inhibition (i.e., high concentrations). We could not use higher concentrations without reducing Na in the solutions below 80 mM, which is required for reasonably high Na-coupled sugar transport (Restrepo and Kimmich 1985).

Second, using uptake of 3OMD-glucose as a positive control, we showed that our method could discriminate mediated uptake. As expected for active transport in viable tissues, we found higher uptake of 3OMD-glucose than L-glucose when measured simultaneously, and uptake of 3OMD-glucose probe was inhibited by unlabeled 3OMD-glucose, but not unlabeled L-glucose (Fig. 1). The tissues appeared undamaged in our microscopic observations; we did not observe the gross damage apparent in everted sleeves of another avian species (Starck et al. 2000).

Our conclusion that L-glucose interaction with sugar carriers is nil is consistent with an earlier finding in Rainbow lorikeets (*Trichoglossus haematodus*) that L-glucuose uptake in vitro was not inhibited by 50 mM D-glucose (Karasov and Cork 1994). In addition, L-glucose absorption in vivo in the house sparrows was not reduced by the presence of either high concentration of 3OMD-glucose (Fig. 3) or luminal nutrients (Fig. 4). Our interpretation of the in vivo findings is consistent with measurements and theoretical modeling of drug absorption that predict, in the presence of a saturable process, a declining fractional absorption with increased substrate concentration (Yu and Amidon 1998) that we did not observe. We conclude that L-glucose absorption is passive in these birds.

The magnitude and modulation of passive absorption of small hydrophilic solutes in normal animals

In two independent experiments we found evidence of enhanced passive absorption in the presence of luminal nutrients, as predicted based on findings of Pappenheimer and coworkers that Na^+-coupled transport of nutrients reversibly increased intestinal permeability to hydrophilic solutes in vivo. Our two experiments used different populations of birds and two different treatment methods (solution gavage and food in the gut) and two analytical methods (measurement by liquid scintillation and by enzymatic spectrophotometry) that could have yielded different results, yet we obtained similar results.

Our results are consistent with our earlier report of substantial (75%) absorption by intact feeding house sparrows of [^{3}H]L-glucose (Caviedes-Vidal and Karasov 1996). We have extended those findings by measuring absorption in the presence/absence of luminal nutrients and finding an enhancing effect of luminal nutrients. Within each of (but not between) Experiment 2 and Experiment 3, the same methodology was used to measure fractional absorption of L-glucose in the presence and absence of luminal nutrients, and absorption was significantly higher in the presence of nutrients, by eight or nine percentage points in both experiments. The similarity in magnitude of response to luminal nutrients occurred despite the differences in how the level of luminal nutrients was increased. Experiment 2 compared sparrows gavaged with solutions containing either mannitol or 3OMD-glucose, and Experiment 3 compared fed and nonfed sparrows.

Two factors may have contributed to the lower fractional absorptions recorded in Experiment 3, compared with Experiment 2. First, sampling of blood began 10 min post-administration of probe in order to reduce stress on the sparrows, but the loss of data at early sampling points (< 10 min) causes an underestimate of at least seven percentage points in fractional absorption (Chediack et al. 2001). Second, the determination of L-glucose by an enzymatic/spectrophotometric method, including the subtraction of a large background signal from exogenous and endogenous D-glucose, is not as precise or as accurate as determination by liquid scintillation (Experiment 2), especially as sample levels approach background. We considered two other factors but doubted that they explained the difference. The luminal osmotic pressures could have differed between the experiments, but in an earlier study we found no significant difference in the fractional absorption of mannitol when it was gavaged in solutions ranging in concentration from 1–1,000 mM (Chediack et al. 2001). Fractional absorption of L-glucose is not overestimated in Experiment 2 due to transfer of radiolabel to compounds other than L-glucose. Essentially all ^{14}C activity in plasma (95%) was associated with L-glucose in these experiments. In another avian species, rainbow lorikeets, no metabolism of L-glucose was observed and the labeled L-glucose was entirely excreted (Karasov and Cork 1994).

Our results are consistent with our earlier report that other small, hydrophilic compounds are absorbed faster and to a greater extent by house sparrows when there are nutrients (e.g., sugar, food) in the gut (Chediack et al. 2003). These findings are consistent with

the solvent-drag hypothesis first proposed by Pappenheimer and coworkers in 1987 (Madara and Pappenheimer 1987; Pappenheimer 1987; Pappenheimer and Reiss 1987). Our study cannot distinguish between two possible mechanisms for the effect of luminal nutrients. It is possible that Na^+-cotransported solutes that are transported across the luminal brush-border effect a specific alteration of the cellular cytoskeleton, resulting in rearrangement of tight-junction complexes and increased paracellular permeability to water and hydrophilic molecules (Pappenheimer and Volpp 1992; Sadowski and Meddings1993; See and Bass 1993; Turner and Madara 1995). It is also possible that increased water flux alone, associated with Na^+-coupled nutrient transport, increased the paracellular flux of L-glucose by solvent drag with no change in the permeability characteristic of the pathway.

Schwartz et al. (1995) suggested that the high fractional absorption of radiolabeled L-glucose in rats was due to mediated transport via the Na-glucose co-transporter SGLT1. We strongly doubt this explanation in the case of house sparrows because our in vitro studies have failed to find evidence that L-glucose transport is mediated and because other monosaccharides such as arabinose and mannitol are absorbed to similar extents as L-glucose in vivo (Chediack et al. 2001). Schwartz et al. (1995) also suggested that increased fractional absorption by rats of L-glucose in the presence of luminal nutrients occurred not because of faster absorption rate at the membrane, but because the slow rate of absorption was extended over a longer time period due to longer digesta retention, or was extended over a greater length of the intestine. We doubt this explanation in the case of house sparrows because our measures of digesta retention time in Experiment 3 failed to find any difference between fed and nonfed sparrows. Also, inspection of the plasma vs. time curve in Experiment 2 (Fig. 3) suggests more rapid absorption of L-glucose (earlier peak) in the presence of 3OMD-glucose than in the presence of mannitol.

Nutritional, biomedical and ecological significance of passive absorption

Our results help explain a feature of whole-animal digestive function in birds, and also highlight features of possible ecological and biomedical significance. As regards the first point, most birds maintain a high digestive efficiency when switched from a low sugar to a high sugar diet, but unlike many mammals that have been tested they do not appear to rely on induction of SGLT1 to achieve this (Karasov 1996). By its nature, a passive absorption process provides a rate of absorption directly related to concentration, and this relationship is enhanced for sugar absorption even more when the apparent permeability through the paracellular path is increased by high sugar concentration, as appears to be the case in house sparrows. Thus, glucose can be absorbed passively at high efficiency even from high sugar meals. Mediated absorption is important for setting up conditions for extensive passive absorption by paracellular diffusion and solvent drag (Madara and Pappenheimer 1987; Pappenheimer 1987; Pappenheimer and Reiss 1987), and it becomes especially important for achieving quantitative extraction at lower glucose concentrations near the end of absorption for a meal. In the case of the house sparrow, the birds typically absorb nearly 100% of glucose in a meal, and passive absorption can account for the absorption of about 75% of this (Caviedes-Vidal and Karasov 1996), which should not be taken as a statement that it actually does. Simultaneous measurement of rates of absorption of D- and L-glucose would be the best way to test that (Chang and Karasov 2001).

While passive absorption appears to provide some advantage, it does not come without certain other costs. A high intestinal permeability, permitting passive absorption, is less selective than a carrier-mediated system. In house sparrows, hydrophilic compounds at least as large as lactulose (MW 342 Da) are absorbed (Chediack et al. 2003). Less discriminate absorption of lumen contents might permit natural toxins to be absorbed from plant and animal material. This vulnerability to toxins could be an important ecological driving force, constraining exploratory behavior and limiting the breath of the dietary niche of wild animals.

The paracellular path could also provide an important route for absorption of water-soluble herbicides, pesticides, and drugs. Knowledge about modulation of this pathway is important for understanding and predicting the rate and extent of their absorption. We think that future whole-animal studies like the present one, testing different probes and different putative modulators of the paracellular pathway (Pappenheimer and Volpp 1992; Sadowski and Meddings 1993; See and Bass 1993; Turner and Madara 1995) will help illuminate the nutritional and pharmacological significance of paracellular absorption.

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