

Absorption of sugars in the Egyptian fruit bat (*Rousettus aegyptiacus*): a paradox explained

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Summary

Two decades ago D. J. Keegan reported results on Egyptian fruit bats (*Rousettus aegyptiacus*, Megachiroptera) that were strangely at odds with the prevailing understanding of how glucose is absorbed in the mammalian intestine. Keegan's *in vitro* tests for glucose transport against a concentration gradient and with phloridzin inhibition in fruit bat intestine were all negative, although he used several different tissue preparations and had positive control results with laboratory rats. Because glucose absorption by fruit bats is nonetheless efficient, Keegan postulated that the rapid glucose absorption from the fruit bat intestine is not through the enterocytes, but must occur *via* spaces between the cells. Thus, we hypothesized that absorption of water-soluble compounds that are not actively transported would be extensive in these bats, and would decline with increasing molecular mass in accord with sieve-like paracellular absorption. We did not presume from Keegan's studies that there is no Na⁺-coupled, mediated sugar transport in these bats, and our study was not designed to rule it out, but rather to quantify the level

of possible non-mediated absorption. Using a standard pharmacokinetic technique, we fed, or injected intraperitoneally, the metabolically inert carbohydrates L-rhamnose (molecular mass=164 Da) and cellobiose (molecular mass=342 Da), which are absorbed by paracellular uptake, and 3-O-methyl-D-glucose (3OMD-glucose), a D-glucose analog that is absorbed *via* both mediated (active) and paracellular uptake. As predicted, the bioavailability of paracellular probes declined with increasing molecular mass (rhamnose, 62±4%; cellobiose, 22±4%) and was significantly higher in bats than has been reported for rats and other mammals. In addition, fractional absorption of 3OMD-glucose was high (91±2%). We estimated that Egyptian fruit bats rely on passive, paracellular absorption for the majority of their glucose absorption (at least 55% of 3OMD-glucose absorption), much more than in non-flying mammals.

Key words: paracellular nutrient uptake, carbohydrate absorption, Chiroptera, Egyptian fruit bat, *Rousettus aegyptiacus*.

Introduction

Frugivorous and nectarivorous bats are known to digest carbohydrate-rich meals relatively rapidly (Keegan, 1977; Winter, 1998) and efficiently (Craik and Markovich, 2000; Korine et al., 1996; Morrison, 1980; Tedman and Hall, 1985), but there are few reports on the mechanisms by which they absorb sugars. All vertebrates are considered to possess intestinal sugar transporters such as the Na⁺-coupled glucose transporter SGLT1 in the apical membrane and GLUT2 in the basolateral membrane (Karasov and Hume, 1997). Karasov and Diamond reported the presence of such mediated D-glucose uptake at the intestinal apical membrane of the New World fruit

bat *Artibeus jamaicensis* (Microchiroptera) (Karasov and Diamond, 1988). However, long before most of these studies, Keegan reported a series of findings on the Egyptian fruit bat (*Rousettus aegyptiacus*, Megachiroptera) that were puzzlingly at odds with the prevailing understanding of how intestinal glucose is absorbed in vertebrates (Keegan, 1977; Keegan, 1980; Keegan, 1982; Keegan, 1984; Keegan and Mödinger, 1979). Keegan's tests for active glucose transport against a gradient by isolated intestine of Egyptian fruit bats were negative in four different tissue preparations, including everted sacs, intestinal rings and isolated, perfused lengths of intestine, even though his simultaneous control experiments with

laboratory rats, using the same methods, were all positive (Keegan, 1980; Keegan, 1984). Keegan also found that D-glucose absorption by the laboratory rat, but not by the Egyptian fruit bat, was significantly inhibited by phloridzin, a specific SGLT1 inhibitor (Keegan, 1982). In addition, Keegan and Mödinger found that the fruit bat's small intestine was relatively more permeable to glucose than that of the rat, the former but not the latter showing significant flux of glucose from the blood into the lumen of the intestine during perfusion studies (Keegan and Mödinger, 1979). In fact, prior to publication of the seminal papers by Pappenheimer and colleagues on passive glucose absorption by paracellular transport across the intestine (Madara and Pappenheimer, 1987; Pappenheimer, 1987; Pappenheimer and Reiss, 1987), Keegan observed that 'it seems necessary to postulate that the rapid glucose absorption from the bat intestine is not through enterocytes, but must occur between these cells' (Keegan, 1984). Further adding to the paradox of rapid and efficient glucose absorption by bats, several biologists have noted that bats tend to have less intestinal tissue than comparably sized non-flying mammals (Klite, 1965; Keegan and Mödinger, 1979), perhaps a weight-saving adaptation to flight.

The possibility that the various *in vitro* techniques used by Keegan grossly underestimated active glucose transport in live bats cannot be ruled out. Therefore, we sought independent evidence for the alternative explanation that substantial passive absorption of glucose occurs in Egyptian fruit bats. We did not presume from Keegan's studies that there is no Na⁺-coupled-mediated sugar transport in these bats, and our study was not designed to rule it out. We highlight Keegan's studies, reported mainly in the form of conference abstracts, to draw attention to a situation in which alternative modes of sugar absorption might be important. In both mammalian and avian species the paracellular route of absorption of water soluble compounds has been visualized using either autoradiography [of radiolabeled polyethylene glycol (Ma et al., 1993)] or confocal laser microscopy [using fluorescein (Chang and Karasov, 2004; Hurni et al., 1993; Sakai et al., 1997)], its molecular size selectivity has been characterized using a series of nonelectrolyte water-soluble probes that differ in molecular dimension (Chediack et al., 2003; Ghandehari et al., 1997; Hamilton et al., 1987), and its charge selectivity has been characterized using relatively inert, charged peptides (Chediack et al., 2006; He et al., 1998). Our goal was to quantify the level of possible non-mediated absorption. We applied *in vivo* pharmacokinetic measures of uptake that we have recently used in studies with birds (Chang et al., 2004; Chediack et al., 2003; Chediack et al., 2001) to fruit bats. In such experiments, carbohydrates that are not catabolized are assayed in serial blood samples taken following either feeding or injection of a solution containing the compounds. Paracellular absorption occurs by diffusion and by solvent drag across intestinal tight junctions secondarily to active sugar and amino acid transport (Pappenheimer and Reiss, 1987), and its magnitude declines with increasing molecular size of the transported solute

because of the pathway's sieve-like qualities (Chediack et al., 2003). Therefore, our test solutions included inert (non-metabolized and not actively transported) carbohydrates of two sizes (L-rhamnose, molecular mass [MM]=164 Da; cellobiose, 342 Da), both of which are commonly used in tests of passive (non-carrier-mediated) intestinal permeability (Cobden et al., 1985; Dinmore et al., 1994; Generoso et al., 2003; Menzies et al., 1999; Saweirs et al., 1985; Travis and Menzies, 1992).

It is conceivable that in experiments such as these, compounds not actively transported may be absorbed at a much slower rate than actively transported D-glucose, but over the entire length of the intestine. Thus, with the extended time of digesta residence in the gut, their absorption could still be fairly complete, resulting in a similar fractional absorption (i.e. absorption efficiency) to that of D-glucose (Schwartz et al., 1995). An elegant approach to resolving this issue is to simultaneously compare, in intact animals, the extent and/or rate of absorption of compounds absorbed passively with D-glucose or analogues that are both actively and passively absorbed. For example, in laboratory rats, the absorption rate of the nonmetabolizable, actively transported 3-O-methyl-D-glucose (3OMD-glucose; MM=194 Da) apparently exceeded that of L-glucose, which is passively absorbed, by approximately 9:1, implying that most glucose was actively absorbed (Uhing and Kimura, 1995). Similar conclusions have been drawn for dogs (Lane et al., 1999; Pencek et al., 2002) and humans (Fine et al., 1993), but in house sparrows we previously found a ratio close to 1:1, suggesting that most glucose absorption was passive (Chang and Karasov, 2004). In order to apply this technique to Egyptian fruit bats, we also included 3OMD-glucose in our test solutions.

We hypothesized that most glucose absorption in Egyptian fruit bats occurs *via* a passive, paracellular pathway as a compensation for lower tissue-specific rates of mediated absorption and possibly also for reduced gut size (Klite, 1965; Keegan and Mödinger, 1979) associated with weight savings for flight. Based on Keegan's earlier findings, we tested three specific predictions: (1) that the extent of absorption (i.e. fractional absorption or bioavailability) of the nonactively transported compounds is relatively high compared with measurements in laboratory rats using similar methods (Lavin et al., 2004); (2) that the extent of absorption of these compounds is inversely related to their MM, consistent with theoretical expectations for the sieving properties of the paracellular pathway (Chediack et al., 2003); and (3) that the rates of absorption of 3OMD-glucose and the non-actively transported compounds are similar, after adjustment for differences in molecular size [the ratio of absorption rates of passive to active probes gives the proportional contribution of paracellular absorption (Chang and Karasov, 2004)].

Materials and methods

Eleven adult Egyptian fruit bats (*Rousettus aegyptiacus*; five male, six non-breeding female; body mass=140.85±5.7 g) were

selected from the colony maintained at the Jacob Blaustein Institutes for Desert Research, Midreshet Ben-Gurion, Israel. The colony is kept in large (5×4×2.5 m) flight cages and is fed an assortment of fruits daily at sunset. Bats were removed from the colony at sunset for experiments, immediately prior to their normal feeding time.

For an index of paracellular (non-mediated) uptake, as well as an indication of the effects of probe size on paracellular uptake, we chose to use L-rhamnose (MM=164 Da) and cellobiose (MM=342 Da) as carbohydrate probes. 3-O-methyl-D-glucose (3OMD-glucose, MM=194 Da), which is absorbed *via* both mediated and paracellular pathways, was also included in the test solutions. Each bat received the solutions (containing all probes simultaneously) both orally and by intra-peritoneal injection, with the treatments separated by at least 2 weeks to ensure complete elimination of the probes. Bats were randomly assigned to sequence of treatment (oral or injection). The curves of plasma marker concentration over time for the two treatments were used to calculate bioavailability and relative rates of absorption, as described below (see Pharmacokinetic calculation of absorption). The solution was orally administered by placing the end of a syringe into the bat's mouth and letting the bat lick and swallow the liquid as the syringe plunger was slowly depressed. It took 20–60 s to administer the entire volume of liquid. For both oral and injection treatments, we measured the volume of liquid given by weighing the syringe before and after administration of the solution. Treatments were given within two hours of sunset, at approximately the beginning of the normal activity period.

The oral and injected solutions were identical in composition, but different in volume; we injected 0.2% of body mass and fed 2% of body mass. Each solution contained L-rhamnose (50 mmol l⁻¹), 3OMD-glucose (50 mmol l⁻¹), cellobiose (140 mmol l⁻¹) and NaCl (30 mmol l⁻¹). NaCl was included in the solution to balance osmolality to approximately 300 mOsm. Inclusion of Na⁺ also provides an essential ion for Na⁺-coupled D-glucose absorption (Brody, 1999), although it is not strictly necessary in this kind of whole-animal study because fruit bats routinely consume low Na⁺ fruits (Arad and Korine, 1993; Nelson et al., 2000; O'Brien et al., 1998; Shanahan et al., 2001; Wendeln et al., 2000) and still absorb nearly all of the sugar (Keegan, 1984; Korine et al., 2004). Additional Na⁺ is secreted into the intestinal lumen together with bicarbonate and diffuses from blood (Brody, 1999).

Following treatment, we took nine serial blood samples of approximately 100 µl each from a wing vein by piercing the vein with a 30G needle and collecting blood into standard heparinized 75 µl capillary tubes (Fisher Scientific, Pittsburg, PA, USA; Hettich Zentrifugen, Tuttlingen, Germany). Sampling times were 0 (pretreatment background), 5, 10, 15, 20, 30, 45, 90 and 120 min after injection, and 0, 10, 15, 20, 30, 45, 90, 120 and 240 min after oral ingestion. Between the first several samples, bats were kept in cotton bags; after 20 min, they were transferred to individual cages between sampling, and after 45 min water was provided *ad libitum*. At the end of a trial, bats were kept overnight in the laboratory in

individual cages, offered food and water *ad libitum*, and returned to the colony in the morning.

Sample analysis

Blood plasma was separated from cells by centrifugation. Plasma mass was determined to ±0.1 mg and samples were deproteinated using a Nanosep 30K omega molecular weight cut-off centrifuge filter (part number OD030C35; Pall Corporation, East Hills, NY, USA). Plasma was initially filtered with 50 µl of double-distilled H₂O (DDW) by centrifugation at 14 000 g for 30 min, followed by rinsing with an additional 100 µl of DDW (14 000 g for 100 min). Samples were subsequently dried at 60°C and stored frozen at -80°C until analysis.

Carbohydrate probes were derivatized by reductive amination with anthranilic acid (2-aminobenzoic acid), following Anumula (Anumula, 1994) and Du and Anumula (Du and Anumula, 1998) with minor modifications, to enable fluorescence detection after separation *via* high performance liquid chromatography (HPLC). Briefly, dried plasma samples were reconstituted with 50 µl distilled H₂O and mixed with 50 µl of anthranilic acid reagent solution. The anthranilic acid reagent consisted of 30 mg ml⁻¹ anthranilic acid and 20 mg ml⁻¹ sodium cyanoborohydride dissolved in a previously prepared solution of 5% sodium acetate 3H₂O and 2% boric acid in methanol. Samples were transferred to a screw-cap glass autosampler vial, tightly capped, and heated at 65°C for 3 h. After cooling to ambient temperature, 300 µl of HPLC solvent A (see below) was added to vials, which were mixed vigorously in order to expel the hydrogen gas evolved during the derivatization reaction.

Carbohydrate derivatives were separated on a Waters Pico Tag[®] C-18 reversed phase HPLC column (3.9×150 mm, 5 µm; part number WAT088131; Waters Corporation, Milford, MA, USA) using a 1-butylamine-phosphoric acid-tetrahydrofuran mobile phase system. The separations were performed at 23°C using a flow rate of 1 ml min⁻¹. Solvent A consisted of 0.2% 1-butylamine, 0.5% phosphoric acid and 1% tetrahydrofuran (inhibited) in HPLC-grade water [18.2 MΩ resistance, produced in-house, further filtered through a 0.45 µm hydrophilic polypropylene membrane filter (GH Polypro; part number 66548; Pall Corporation), or purchased] and solvent B consisted of equal parts solvent A and HPLC-grade acetonitrile. Table 1 lists the gradient elution protocol used for the separation.

Derivatives of carbohydrate probes in samples and standard solutions were detected with a Perkin-Elmer 650-10LC fluorescence spectrophotometer (Perkin-Elmer Life and Analytical Sciences, Boston, MA, USA) with the following settings: excitation wavelength 230 nm, slit width 10 nm; emission wavelength 425 nm, slit width 5 nm; sensitivity=1; 'normal' setting for lamp mode, photomultiplier gain and response time. Limits of detection for all probes in water were 1–2 ng loaded onto the HPLC column. All derivatization reagents and HPLC solvents were obtained from Sigma-Aldrich (St Louis, MO, USA).

Table 1. HPLC gradient protocol for carbohydrate probe derivative separation*

Time (min)	Solvent A (%)	Solvent B (%)
0 (initial conditions)	90	10
5.5	90	10
6	80	20
12	66	34
13 (wash)	0	100
16	0	100
18 (equilibrate)	90	10
23 (end time)	90	10

*See Materials and methods for solvent composition, flow rate and column type. All changes in proportional solvent composition were linear over time.

Pharmacokinetic calculation of absorption

The plasma concentrations of each probe, C (ng probe mg^{-1} plasma), were plotted as a function of sample time, t (min). The amounts of the various probes absorbed were calculated from areas under the post-absorption and post-injection plasma curves (AUC=area under the curve of plasma probe concentration *versus* time). 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 $F=(\text{AUC}_{\text{oral}}/\text{dose}_{\text{oral}})/(\text{AUC}_{\text{injection}}/\text{dose}_{\text{injection}})$. 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 $t=x$ to $t=\infty$ was calculated as $\text{AUC}_{x \rightarrow \infty} = C_t$ (at $t=x$)/ K_{el} . The total $\text{AUC}_{0 \rightarrow \infty}$ was obtained by summing the two areas. The parameter K_{el} (min^{-1}) is the elimination constant for removal of the probe from plasma, which was estimated as the slope of the last two log-transformed plasma concentrations as a function of sample time.

Statistical analyses

Numerical results are given as means \pm s.e.m. (N =number of animals unless otherwise indicated). Fractional absorption (F) values for probes were arcsine-square root transformed prior to statistical comparisons (Sokal and Rohlf, 1995). Repeated measures analysis of variance (RM-ANOVA) was used to test for differences in F among probes, with Tukey's honest significant difference (HSD) post-hoc contrasts as appropriate, and paired t -tests were used to compare terminal slopes of post-gavage and post-injection relationships. Mono- and bi-exponential elimination models' fit to semi-log plots of probe concentration *versus* time after injection were compared with F -tests (Motulsky and Ransnas, 1987).

Results

When the bats were fed the solution with L-rhamnose, cellobiose and 3OMD-glucose, the mean concentration of all the carbohydrates in the plasma peaked at ~ 20 min and then

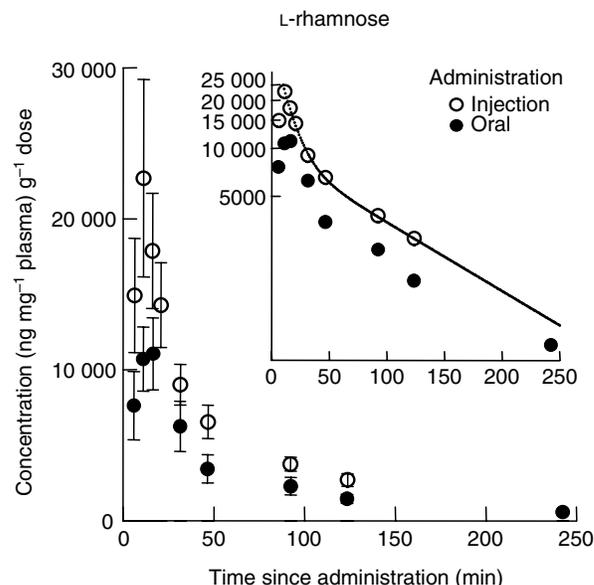


Fig. 1. Plots of mean (\pm s.e.m.) plasma L-rhamnose concentration *versus* time since its oral or injected administration into Egyptian fruit bats ($N=11$). Each concentration (ng mg^{-1} plasma) was normalized to the dose administered to the bat. The inset shows the mean values on a semi-log plot. The line through points from the injection trial is the nonlinear fit to the biexponential model: $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ (see Materials and methods, and Table 2 for derived parameters). The line is extrapolated beyond the data to permit visual comparison with the data from the oral administration trial.

declined exponentially (Figs 1–3). When the solution was injected, blood values for all the carbohydrates peaked at around 10 min and declined exponentially thereafter. Semi-log plots of injection data after the peak (insets of Figs 1–3) were significantly better fit by a model of bi-exponential than mono-exponential decline for L-rhamnose ($F_{2,3}=102$, $P<0.005$), cellobiose ($F_{2,2}=58$, $P<0.025$) and 3OMD-glucose ($F_{2,3}=14.8$, $P<0.05$) (r^2 values for the three carbohydrates were all >0.999). The parameters from the bi-exponential fits (Table 2) were subsequently used to calculate the time course of absorption (below). When comparing the terminal slopes of the log-transformed injection data with those of the oral ingestion data, the terminal slopes based on the last two time points were not significantly different for L-rhamnose (paired $t_{10}=1.4$, $P=0.18$) or cellobiose ($t_{10}=1.14$, $P=0.28$). For 3OMD-glucose, the terminal slopes post-ingestion were significantly less steep than post-injection (paired $t_{10}=3.92$, $P=0.003$). We suspect that in this latter case of 3OMD-glucose, which is transported by mediated as well as paracellular mechanisms, the terminal slope may have been influenced by slower probe turnover from a secondary compartment probably associated with intestinal or hepatic tissue.

It is apparent from visual comparison of areas under the curve (AUC) of the oral and injection administrations (Figs 1–3), that fractional absorption (bioavailability) was least for cellobiose. However, although there is merit in visual

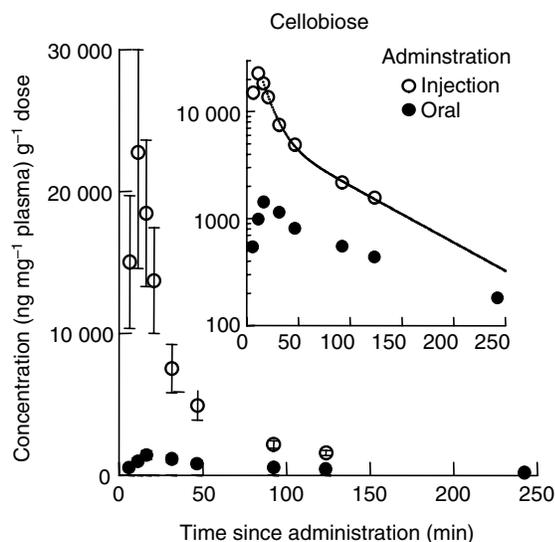


Fig. 2. Plots of mean (\pm s.e.m.) plasma cellobiose concentration *versus* time since its oral or injected administration into Egyptian fruit bats ($N=11$). Each concentration (ng mg^{-1} plasma) was normalized to the dose administered to the bat. The inset displays the mean values on a semi-log plot. The line through points from the injection trial is the nonlinear fit to the biexponential model: $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ (see Materials and methods, and Table 1 for derived parameters). The line is extrapolated beyond the data to permit visual comparison with the data from the oral administration trial.

inspection of the patterns in these plots because it affords the reader a simple and direct way to evaluate the data, more quantitative and sometimes less intuitive patterns emerge from calculations of fractional absorption (the quotient of $\text{AUC}_{\text{oral}}/\text{AUC}_{\text{injection}}$). The relatively low absorption of cellobiose was confirmed ($F=0.22\pm 0.04$, $N=11$), but the calculation also showed that absorption of 3OMD-glucose ($F=0.91\pm 0.02$) was significantly higher than that for L-rhamnose ($F=0.62\pm 0.04$). By RM-ANOVA, all means differ significantly with $P<0.001$ in each case. Fractional absorption of paracellular probes declined with increasing MM (Fig. 4).

The time over which absorption of the carbohydrates occurred was calculated following Loo and Riegelman (Loo and Riegelman, 1968), using kinetic constants derived from the injection plots (Table 2) and the mean plasma concentrations following oral administration of each compound (Figs 1–3). As an index of the rate of absorption, we used the time to absorb 50% of the probe in question (τ); the absorption of 3OMD-glucose (τ between 6 and 11 min) was slightly faster than that for L-rhamnose (τ between 11 and 16 min) (Fig. 5A). By 2 h post-ingestion, the absorption of all three compounds was 80–88% of the quantity ultimately absorbed.

In order to estimate how much 3OMD-glucose was passively absorbed, we assumed that the rate of absorption of L-rhamnose can serve as a proxy for the passive absorption of 3OMD-glucose, once adjusted for the small difference in MM. Because diffusion in water declines with $\text{MM}^{1/2}$ (Smulders and Wright, 1971), each value of L-rhamnose absorption was decreased by

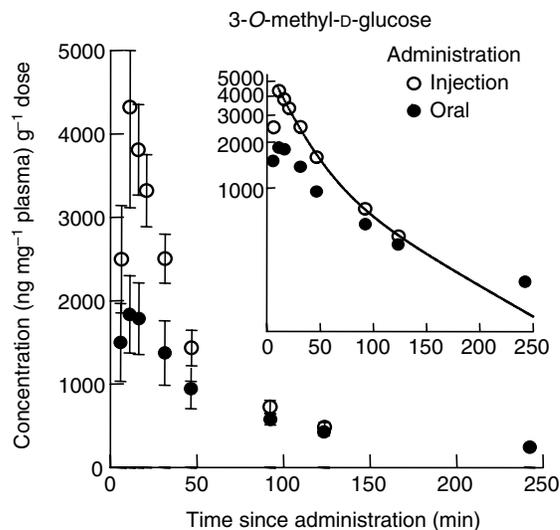


Fig. 3. Plots of mean (\pm s.e.m.) plasma 3-O-methyl-D-glucose concentration *versus* time since its oral or injected administration into Egyptian fruit bats ($N=11$). Each concentration (ng mg^{-1} plasma) was normalized to the dose administered to the Egyptian fruit bat. The inset displays the mean values on a semi-log plot. The line through points from the injection trial is the nonlinear fit to the biexponential model: $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ (see Materials and methods, and Table 1 for derived parameters). The line is extrapolated beyond the data to permit visual comparison with the data from the oral administration trial.

8% [$100 \times (194^{1/2} - 164^{1/2}) / 194^{1/2}$]. Assuming that the absorption of 3OMD-glucose represents the sum of passive and mediated absorption, the ratio of the amounts absorbed (L-rhamnose/3OMD-glucose) indicates the proportion of 3OMD-glucose absorption that occurs *via* the passive pathway. The ratios exceeded 0.6 (range 0.6–0.88) at every sampling time, apparently indicating that at least 60% of 3OMD-glucose absorption was passive at whatever sampling time one chose to use in calculating apparent absorption rate (i.e. from zero to 5 min or zero to 2 h).

Discussion

We found that Egyptian fruit bats have high fractional absorptions of paracellular probes that decline with increasing probe size, which is consistent with theoretical expectations for the sieving properties of the paracellular pathway (Chediack et al., 2003). Our simultaneous measurements of 3OMD-glucose and L-rhamnose (as a proxy for non-mediated uptake of glucose) suggest that Keegan's explanation for the low mediated glucose uptake rates he measured was correct, namely that Egyptian fruit bats absorb most glucose *via* a passive, paracellular pathway rather than a mediated, transcellular one.

With methods comparable to those used for *R. aegyptiacus*, we also measured the paracellular absorption of L-rhamnose and lactulose (an isomer of cellobiose that is also absorbed paracellularly) in Sprague-Dawley laboratory rats (Lavin et al., 2004). Comparison of fractional absorptions between rats and

Table 2. Summary of bi-exponential fits of plasma probe concentrations versus time from injection/elimination experiments shown in Figs 1–3*

Parameter	Probe		
	L-rhamnose (Fig. 1)	Cellobiose (Fig. 2)	3OMD-glucose (Fig. 3)
A (ng mg ⁻¹ plasma g ⁻¹)	40 000±3000	56 096±15 416	4983±542
α (min ⁻¹)	0.088±0.0007	0.09±0.019	0.412±0.0074
B (ng mg ⁻¹ plasma g ⁻¹)	9237±696	6957±1772	1423±707
β (min ⁻¹)	0.00995±0.007	0.0122±0.0023	0.0092±0.0036

*The model was $C_t = Ae^{-\alpha t} + Be^{-\beta t}$. Mean concentrations for each probe (based on measurements in eight bats) were fit to the model.

R. aegyptiacus by two-factor ANOVA (species and carbohydrate probe as factors) points to a highly significant difference between the two species (species: $F_{1,32}=57.2$, $P<0.0001$; probe: $F_{1,32}=59.4$, $P<0.0001$), with *R. aegyptiacus* having greater paracellular absorption than rats for both probes (Fig. 4). This is consistent with the conclusions of Keegan et al. that the intestines of *R. aegyptiacus* are more permeable than those of laboratory rats (Keegan et al., 1979).

In simultaneous measurements of absorption of actively transported D-glucose or 3OMD-glucose and passively transported L-glucose (Ikeda et al., 1989) in rats (Uhing and Kimura, 1995), dogs (Lane et al., 1999; Pencek et al., 2002) and humans (Fine et al., 1993), D-glucose or its analog 3OMD-glucose were absorbed approximately ten times faster than L-glucose, implying that more than 90% of glucose absorption was mediated. The high fractional absorption of L-rhamnose relative to 3OMD-glucose in the present study implies that a significant proportion of total glucose uptake is paracellular. However, simply comparing fractional absorption for actively transportable versus nonactively transportable compounds could be misleading (Schwartz et al., 1995). Suppose that 3OMD-glucose is actively absorbed at a high rate in the proximal portion of the intestine, whereas L-rhamnose is passively absorbed at a very slow rate. The fractional absorption of L-rhamnose could be fairly complete, despite its slow absorption rate, if absorption occurs over the entire length of the intestine and over the entire time of digesta residence. We do not think that this explanation applies to *R. aegyptiacus*. L-rhamnose absorption, normalized to $MM^{1/2}$ (to account for size-dependent differences in diffusion of the probe), did not seem slow or prolonged compared with that for 3OMD-glucose. These probes had apparent absorption rates similar to each other throughout all the sampling time points (Fig. 5A). The ratio of diffusion normalized L-rhamnose to 3OMD-glucose apparent absorption rates exceeded 0.6 (range 0.6–0.88) at every sampling time, indicating that at least 60% of total 3OMD-glucose absorption was passive (Fig. 5B). It is important to note as a caveat, however, that 3OMD-glucose is handicapped relative to D-glucose. The affinity of the glucose transporters for 3OMD-glucose is lower than for D-glucose (Ikeda et al., 1989; Kimmich, 1981), so the former is an imperfect substitute for the latter and its use will give results

that slightly underestimate the relative contribution of mediated glucose absorption (McWhorter et al., 2005). This concern, of course, applies to all studies that use 3OMD-glucose for similar measurements (e.g. Pencek et al., 2002; Uhing and Kimura, 1995).

Because their rates of absorption were similar (see above and Fig. 5A), direct comparison of the fractional absorptions of L-rhamnose and 3OMD-glucose can be used to provide corroborating evidence for our estimate of the proportional contribution of paracellular to total glucose uptake. Because this additional comparison is based on bioavailability data and not apparent rates of absorption, it is much less likely to be biased by differences in affinity for SGLT1 between 3OMD-glucose and D-glucose. Bioavailability of radiolabeled 3OMD-glucose and D-glucose in birds obtained using an identical pharmacokinetic protocol to that used in this study were not significantly different from each other (McWhorter et al., 2005) (T.J.M., W.H.K. and A. K. Green, unpublished data). The fractional absorption of passively absorbed carbohydrates declines with increasing MM of the probe (Fig. 4) (see also Chediack et al., 2003). Besides the fact that diffusion coefficients decline with increasing $MM^{1/2}$, for which we have already corrected, the paracellular space acts like a sieve and discriminates according to molecular size (Chang et al., 1975; Friedman, 1987). The direct comparison of the fractional absorptions of L-rhamnose ($F=0.62$) and 3OMD-glucose ($F=0.91$) might be adjusted by decreasing the value of L-rhamnose using the slope of the relation between fractional absorption and MM (Fig. 4) to estimate the effect of the 30 Da difference in MM between the two probes. Thus, the diffusion coefficient corrected fractional absorption of L-rhamnose would be reduced by a further 0.066 (i.e. the product of 30 Da and 0.0022/Da). When normalized to the fractional absorption of 3OMD-glucose, this indicates that at least 55% of 3OMD-glucose uptake is nonmediated, supporting our conclusion that a significant proportion of total glucose absorption in the Egyptian fruit bat occurs by the paracellular pathway. We thus conclude that the paracellular component of glucose absorption in the Egyptian fruit bat is much higher than has been measured in non-flying mammals where this kind of calculation has been made (see above).

In a separate study on the great fruit bat (*Artibeus literatus*),

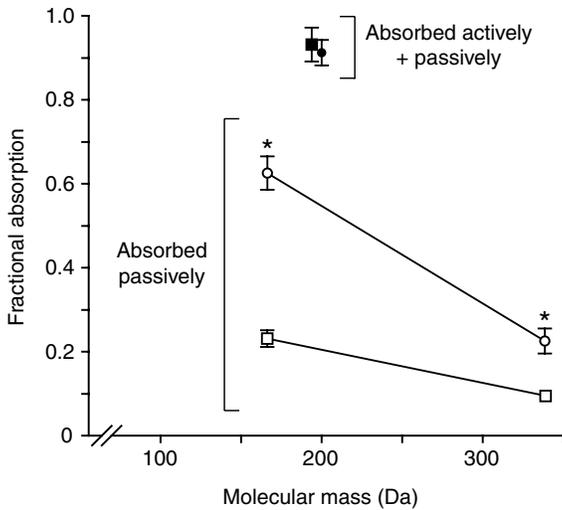


Fig. 4. The fractional absorptions of three carbohydrate probes in Egyptian fruit bats, *Rousettus aegyptiacus* (circles, $N=11$) and Sprague-Dawley laboratory rats [squares, $N=6$; rat data from Lavin et al. (Lavin et al., 2004)]. 3-OMD-glucose (194 Da; closed symbols) is absorbed both actively and passively; L-rhamnose (164 Da) and cellobiose (342 Da) are absorbed passively (open symbols). For rats, lactulose (342 Da), an isomer of cellobiose, was used as a probe instead, and was assumed to behave similarly to cellobiose. Asterisks indicate statistically significant differences ($P < 0.05$) between rats and *R. aegyptiacus* (see main text); error bars are ± 1 s.e.m. (some error bars are smaller than the symbols). Both species show high absorption of 3-OMD-glucose, and both show decreasing absorption of the passively absorbed probes as probe size increases; however, passive absorption by *R. aegyptiacus* was significantly higher than that by rats for both passively absorbed probes.

a microchiropteran frugivore, we also found relatively high bioavailability of water-soluble compounds that are not actively transported, and calculated that most 3OMD-glucose absorption that was measured (>70%) could have been passive (Caviedes-Vidal et al., 2004). This suggests that high paracellular absorption may be a general pattern among fruit-eating bats, which may have less intestinal tissue than similarly sized non-flying mammals (Klite, 1965; Keegan and Mödinger, 1979) in spite of high energetic demands. At least three mechanisms may explain differences between bats and other mammals in the importance of paracellular uptake. (1) Paracellular permeability might be increased by a larger effective pore radius in the tight junctions between enterocytes, as a result of differences in the number and complexity of protein strands, and the composition of claudins and other proteins that create the sieving effect (Chang et al., 1975; Chediack et al., 2003). However, the pattern of decline in fractional absorption with increasing probe size in *R. aegyptiacus* is similar to that in other mammals (Fig. 4), suggesting that differences among groups in effective pore size may be small. (2) Higher water flux through the tight junctions would increase solute permeation by increased solvent drag (Pappenheimer, 1990; Pappenheimer and Reiss, 1987). We have no information on

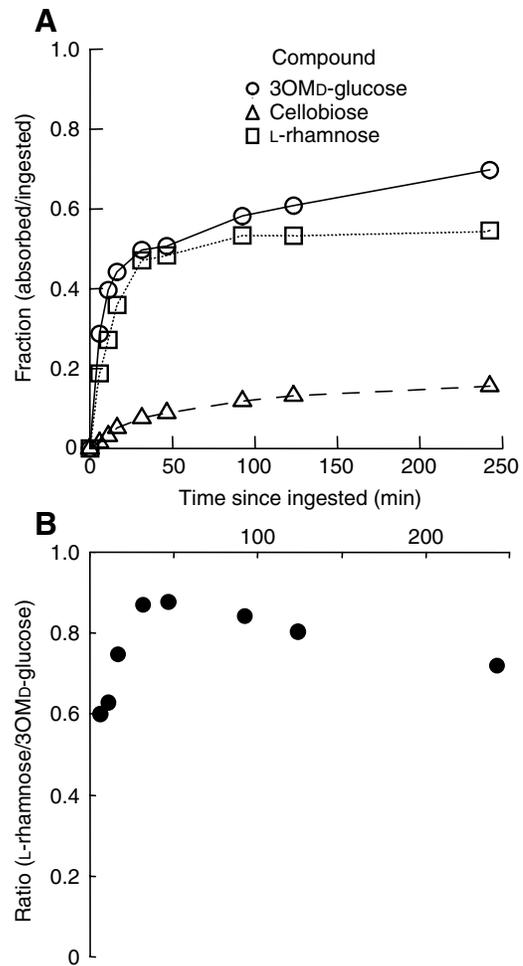


Fig. 5. (A) Cumulative absorption versus time since ingestion of 3-O-methyl-D-glucose (3OMD-glucose; open circles, solid line), cellobiose (open triangle, broken line) and L-rhamnose (open square, dotted line) by Egyptian fruit bats. (B) The ratios of apparent absorption of L-rhamnose and 3OMD-glucose were calculated from the cumulative amounts absorbed in Fig. 5A. Assuming that absorption of L-rhamnose is passive, whereas the absorption of 3OMD-glucose represents the sum of passive plus mediated absorption, the ratio of the apparent absorption (L-rhamnose/3OMD-glucose) indicates the proportion of 3OMD-glucose absorption that occurs via the passive pathway. These data suggest that at least 60% of 3OMD-glucose absorption was passive, whatever time point one chooses to use in calculating apparent absorption rate (i.e. from zero to 5 min or zero to 2 h).

water flux in bats to compare with other mammals. However, Makanya et al. reported that the intercellular spaces between enterocytes in the epauletted Wahlberg's fruit bat (*Epomophorus wahlbergi*) were relatively large, consistent with significant paracellular fluid absorption (Makanya et al., 2001). They also noted that the lateral cell membranes were 'modified into elaborate, long and tortuous interdigitating cytoplasmic processes' that greatly increased surface area. Amplified lateral membrane surface area and a preponderance of mitochondria in the adjacent cytosol may increase capacity

for sodium or nutrient transport into intercellular spaces in fruit bats (Makanya et al., 2001), creating an osmotic gradient that draws water and solutes across the epithelium, driving paracellular absorption (Pappenheimer and Reiss, 1987). (3) There is some evidence that bats have a higher ratio of villous area relative to nominal intestinal surface area (sometimes called the surface enlargement factor or SEF) when compared with non-flying mammals. Two research groups have compared similar-sized bat and non-flying mammal species using measurements made with uniform methods (Barry, 1976; Makanya et al., 1997; Mayhew and Middleton, 1985). In both cases the SEF in the bat exceeded that in its non-flying counterpart by $\geq 59\%$. Makanya et al. hypothesized that the larger villous surface area in bats compared with non-flying mammals may be because of an increase in the number of enterocytes (Makanya et al., 1997). A corresponding increase in the number of cell junctions (i.e. tight junction surface area), the pathway for paracellular transport, could partly account for relatively high paracellular absorption in *R. aegyptiacus*.

From an evolutionary perspective, high intestinal permeability to hydrosoluble compounds could convey both costs and benefits, which may explain some of the variation among mammalian species. On the one hand, high intestinal permeability that permits paracellular absorption is likely to be less selective than carrier-mediated nutrient absorption. This might result in absorption of toxins from plant or animal material in the intestinal lumen (Diamond, 1991); an evolutionary cost. On the other hand, Pappenheimer suggested that passive absorption may be selectively advantageous because it requires little energy (Pappenheimer, 1993). These opposing costs and benefits can lead to variation in intestinal permeability to hydrosoluble biochemicals among species. For fruit bats, susceptibility to hydrosoluble toxins resulting from high intestinal permeability could significantly increase metabolic costs for detoxification, and thus could be an important selective force limiting the breadth of the dietary niche. Determining whether strong dependence on paracellular nutrient absorption is a general pattern in flying mammals (i.e. associated with weight-saving reductions in gut tissue) or a function of diet must await similar measurements in bats with other diets (insects, nectar) that will facilitate a phylogenetically corrected analysis including gut morphometric data. However, recent results in birds suggest that high paracellular nutrient absorption holds across a broad range of diet types (reviewed by McWhorter, 2005).

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