Test, Rejection, and Reformulation of a Chemical Reactor–Based Model of Gut Function in a Fruit-Eating Bird

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ABSTRACT

We explored modulation of retention time in cedar waxwings (*Bombycilla cedrorum*) by feeding them diets varying in hexose concentration. Our goals were to (1) test three predictions of a chemical reactor–based model of how guts might respond optimally to diet shifts; (2) determine whether modulation of retention time can occur quickly, thereby facilitating rapid changes in diet; (3) tease apart the relative influence of ingestion rate and nutrient concentration on retention time; and (4) examine the degree of axial mixing in the intestine and its relationship with retention time. The model's predictions were rejected: mean retention time did not decrease, ingestion rate did not increase, and glucose assimilation efficiency did not decrease with increased hexose concentration of the diet. Instead, birds displayed maximal intake rate at intermediate sugar concentration, and mouth to cloaca mean retention times increased with hexose concentration. Significant modulation of retention time occurred quickly, within 3 h of exposure to a different diet. Birds did equally well in terms of total energy assimilated on diets differing 3.3-fold in hexose concentration (from 500 mmol/L to 1660 mmol/L) but showed reduced intake when fed food with low hexose concentration (110 mmol/L). Far more variation in retention time was explained by direct effects of ingestion rate than by direct effects of hexose concentration. Finally, a gut dispersion index that measured degree of axial mixing was positively correlated with mean retention time, indicating that higher retention times are accompanied by increased axial mixing. We propose a modification of the assumptions of the original model. The resulting "osmotic constraint" model better captures the interaction between feeding rate and digestive function in fruit-eating birds.

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Introduction

Digestive physiology is important for ecologists to consider because it determines how efficiently and quickly food is processed, which in turn may influence foraging behavior, diet selection, and, ultimately, rates of growth and reproduction (Karasov 1990). A central issue is the extent to which digestive processing influences diet versus the extent to which diet influences digestive processing.

The effect of digestive processing on diet depends on a food item's profitability, which is largely determined by digestive efficiency (the proportion of ingested food that is digested and absorbed) and the amount of the item consumed per unit time. These parameters are presumably determined by retention time in the gastrointestinal tract, rates at which nutrients in food are hydrolyzed and absorbed (i.e., reaction rates), and concentration of nutrients in food (Levey and Karasov 1992; Karasov 1996).

The effect of diet on digestive efficiency is influenced by how much modulation can occur in retention time, reaction rate, and digesta volume, given dietary changes in nutrient concentration and composition. If one or more of these parameters is static or set at an extremely low level, constraints on diet can result (Martínez del Rio and Stevens 1989). But if these processes are plastic, their modulation can increase profitability of the food type that brought about modulation (e.g., Levey and Karasov 1992). Thus, understanding digestive modulation is key to understanding why animals eat what they do.

The interplay of digestive parameters is complex. Modulation of retention time, for example, will influence digestive efficiency but only if no compensatory changes in reaction rates or volume occur and if retention time before modulation was not excessively long. Recently, such interactions have been made explicit through modeling of guts as chemical reactors (Penry and Jumars 1987; Martínez del Rio et al. 1994; Karasov and Cork 1996). Predictions of these models provide a first step in determining how guts function and in deciphering the limits of modulation.

Frugivorous birds offer an exceptionally straightforward system for testing models of digestion because their guts are simple in structure and their food simple in chemical composition,

often consisting mainly of easily absorbed hexoses (Martínez del Rio et al. 1992; Witmer 1996). Also, all frugivorous birds tested thus far show nonspecific, little, or no modulation of biochemical aspects of digestion, such as glucose and amino acid transporter activity and disaccharidase activity (Levey and Karasov 1992; Afik et al. 1995; Martínez del Rio et al. 1995; Karasov et al. 1996).

We conducted feeding trials with cedar waxwings (*Bombycilla cedrorum*), one of the most exclusively frugivorous birds in North America (Witmer 1996). We presented captive birds with five diets spanning a 15-fold range of hexose concentration and recorded consumption and defecation patterns. Our first goal was to test three predictions of a model of optimal gut function proposed specifically for frugivores by Martínez del Rio and Karasov (1990). The basic model is outlined in Figure 1, and its reactor theory approach is described in the following section. We list its predictions along with page numbers in Martínez del Rio and Karasov (1990), where each is derived and explained in detail. We also summarize crucial assumptions that underlie each prediction.

The predictions can be divided into two broad classes: those that focus on the configuration of the gastrointestinal tract and those that focus on its performance. Our main prediction about the configuration of the frugivore gastrointestinal tract was that the excretion pattern of a marker fed in a pulse will be a smooth, negative exponential shifted to the right of time zero (prediction 1; p. 630). This prediction assumes that the digestive tract of fruit-eating birds is analogous to a completely stirred tank reactor in series with a plug-flow reactor (see below).

The primary prediction about the performance of the intestine was that intestinal mean retention time will decrease with increased hexose concentration of the diet (prediction 2; pp. 626–627). A corollary of this prediction is that the food intake rate will increase with sugar concentration because, for guts or reactors of fixed volume, mean retention time equals the ratio of intestinal volume and intake rate. The main assumptions for prediction 2 are that waxwings feed primarily on a hexose diet and that most hexose absorption is passive. These assumptions have both physiological and ecological support. Hexoses appear to be absorbed in waxwing intestines primarily by a passive, and presumably paracellular, pathway (D. Levey, unpublished data), and the fruits encountered by waxwings in the wild contain the hexoses glucose and fructose as their primary energy-providing nutrients (Martínez del Rio et al. 1992; Witmer 1996). Prediction 3 was that assimilation efficiency of glucose will decrease with increasing hexose concentration (p. 627). This prediction is a direct corollary of the assumptions described above for prediction 2. These three predictions are depicted graphically in Figures 1 and 2.

The predictions posed above were recently tested and rejected for rainbow lorikeets (*Trichoglossus haematodus*), a nectarivore (Karasov and Cork 1996). Karasov and Cork (1996) found that intake decreased and assimilation efficiency remained invariant

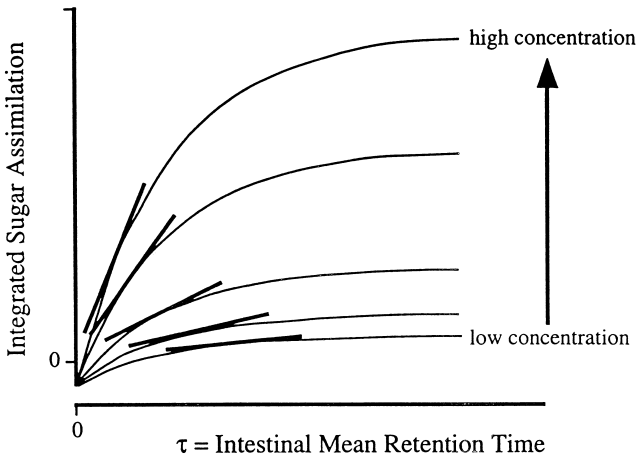

Figure 1. Curves relating the amount of sugar assimilated minus the cost of acquiring a gutful of food to the mean time that digesta is retained in the intestine (mean retention time = τ; modified from Martínez del Rio and Karasov 1990). The following assumptions were used to construct the predictions derived from these curves. (1) Absorption is the primary reaction determining assimilation, and thus assimilation rates follow first-order kinetics. (2) The cost of feeding increases as a linear function of volumetric intake and is the same for food of different hexose concentrations. Assimilation curves are ranked for food of increasing sugar concentration. When curves become flat, hexose assimilation is complete and assimilation efficiency is 100%. Optimal mean retention time can be estimated by the point at which a straight line from the origin is tangent to the integrated sugar assimilation curve. Note that this model predicts decreased optimal intestinal mean retention times as sugar concentration in food increases.

with increased sugar concentration in food. The first part of our study builds on Karasov and Cork's (1996) study by using a frugivorous species and by incorporating more hexose concentrations, spanning a much greater range than those used in their study. Because our results, like those of Karasov and Cork (1996), were not those predicted, we shifted our emphasis toward modifying the model.

Other goals of this study were (1) to determine whether modulation of retention time can occur quickly, thereby facilitating rapid changes in diet; (2) to examine nonphysiological mechanisms underlying variation in gut retention time; and (3) to determine whether longer retention times are accompanied not only by slower flow of ingesta but also by a different pattern of flow. The first of these objectives is important to our understanding of dietary flexibility in birds; why some birds have broad diets and others have narrow diets. The second is justified by the widespread view among physiologists that retention time is largely determined by hormonal and neural reflexes dictated by luminal concentrations of nutrients (Duke 1986). Our study presents an alternative but not mutually exclusive view that variation in retention time can be tightly predicted by variation in ingestion rate, which also is influenced

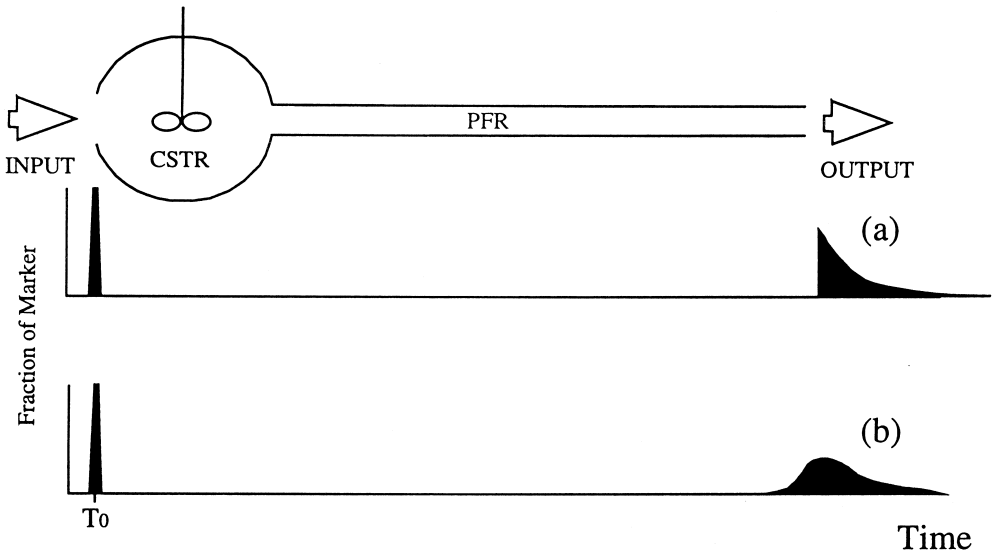

Figure 2. Marker input and output distributions (left and right sides of *x*-axes, respectively) for a chemical reactor consisting of a completely stirred tank reactor (*CSTR*, analogous to a bird's stomach) and a plug-flow reactor (*PFR*, analogous to a bird's intestine). Input is at time zero, T_0. The marker excretion distribution of a reactor with no mixing along the plug-flow reactor is a negative exponential shifted to the right (*a*). Mixing along the plug-flow reactor's length produces a more symmetrical and less leptokurtic marker-output distribution (*b*).

by nutrient concentration (Van Soest 1994; Witmer 1994). By using path analysis, we attempt to tease apart the contributions of hexose concentration and ingestion rate to retention time. The third objective is met by analyzing the shapes of marker excretion curves representing a wide range of retention times. By doing so, we hope to decipher different patterns of ingesta flow and gain insight into how to modify the general model of gut function described above.

Background on Chemical Reactor Theory

We used chemical reactor theory to diagnose the configuration of the gastrointestinal tract and the pattern of digesta flow within it (Penry and Jumars 1987; Martínez del Rio et al. 1994). After an input pulse of an inert marker, reactors with different configurations show characteristic output frequency distributions of the marker. For example, the gastrointestinal tract of fruit- and nectar-eating birds is often assumed to consist of a completely stirred tank reactor (analogous to a bird's crop and stomach) in series with a plug-flow reactor (analogous to a bird's intestine; Martínez del Rio and Karasov 1990; Martínez del Rio et al. 1994; Karasov and Cork 1996). The output distribution that characterizes this configuration is a negative exponential shifted to the right (Fig. 2*a*). This expected distribution relies on the simplifying assumption that the plug-flow section of the reactor (i.e., the intestine) shows little longitudinal mixing relative to axial advective transport (Penry and Jumars 1987; Penry 1989). This assumption is likely false; two types of mixing occur (described below). At issue is how much mixing occurs and under what conditions.

In vertebrates, the small intestine typically exhibits two types of motions that result in mixing: segmentation and peristalsis or antiperistalsis. Segmentation is the most common motion and consists of stationary contraction and relaxation of intestinal segments with little apparent movement of digesta toward the colon. After most of the meal has been digested, the segmenting contractions cease and are replaced by a directional pattern of peristaltic activity that moves undigested material to the colon. In addition to segmentation and peristalsis, many animals exhibit antiperistaltic movements and intestinal refluxes that propel digesta orally (Duke 1986). Both segmentation and antiperistalsis increase the longitudinal mixing of digesta along the intestine and thus invalidate simplifying assumptions inherent in models of guts as chemical reactors. The effect of mixing due to segmentation and antiperistalsis on frequency distributions of marker excretion is that the leptokurtic and highly asymmetrical curve expected in an ideal, completely stirred tank reactor in series with a plug-flow reactor becomes more symmetrical and less sharply peaked (platykurtic) as longitudinal mixing increases (Fig. 2*b*; Levenspiel 1972).

Animals can modulate retention time by increasing intensity of peristalsis, and thus the rate at which digesta is driven aborally, and/or by changing duration of segmentation and frequency of antiperistalsis. Determining the extent to which the shape of a marker excretion distribution differs from that of a

nonmixing model can yield insights about the relative importance of segmentation, antiperistalsis, and peristalsis as determinants of the time that digesta spends in the gut. For example, if frequency distributions of marker excretion become more mound shaped (platykurtic and symmetrical) with increased retention times, increased longitudinal mixing (presumably due to increased segmentation and antiperistalsis relative to axial transport) can be inferred. In contrast, if the shape of a bird's retention time distribution does not change as retention times increase, one can conclude that longer retention times are solely a result of slower flow with no change in flow patterns. We compared curves associated with short and long retention times both qualitatively and quantitatively (via calculations of kurtosis and skewness). Our use of quantitative indices of mixing (kurtosis and skewness) to interpret marker excretion distributions is an extension of the pioneering work of Penry (1989), who used qualitative examination of frequency marker excretion distributions to diagnose gut configuration of deposit-feeding invertebrates. We emphasize that we examined marker excretion distributions not only to determine how much the behavior of waxwing guts deviates from that of ideal reactors but also to generate hypotheses about patterns of digesta flow and the mechanisms that lead to them.

Material and Methods

Cedar Waxwings

Waxwings were captured in commercial blueberry fields near Gainesville, Florida. They were maintained on a diet of mashed bananas and soy protein, provided ad lib. with water, and housed separately in 0.5 × 0.5 × 0.5-m cages behind one-way mirrors. Day length and temperature were kept constant (14L : 10D, 23°C). We pulled plastic sheeting from a roll through slots in the back and front of each cage and then under the mirror. This design allowed us to observe the birds and collect defecations with limited disturbance. In general, the birds seemed oblivious to our presence. When we started experiments, birds had been in captivity for 5 mo and appeared in good health.

Retention Time

We made artificial fruits containing equal amounts of glucose and fructose plus 2% agar. These fruits are processed similarly to real fruits by waxwings (Levey and Grajal 1991). Fruits eaten by waxwings typically contain a 1 : 1 ratio of glucose and fructose and relatively little protein or fat (Martínez del Rio et al. 1992; Witmer 1996). Soluble carbohydrate concentrations in North American fruits vary from 2% (ca. 110 mmol/L) to 41% (ca. 2,270 mmol/L; White and Stiles 1985; Witmer 1994). We made five fruit types, roughly spanning the range of hexose concentration in natural fruits: 110 mmol/L (2%; g hexose/[g hexose + g water]), 500 mmol/L (9%), 890 mmol/L (16%), 1,280 mmol/L (23%), and 1,660 mmol/L (30%). Order of presentation was randomized day to day; all birds on a given day received the same randomly determined concentration. On the day of a trial, birds were given artificial fruits early in the morning and allowed to feed for approximately 2 h. They were then intubated (force fed) with 100 μL of a solution matching the concentration of glucose and fructose in their diet that day and also containing 222 kBq ^{14}C sodium ferrocyanide (^{14}C FeCN; New England Nuclear Research Products, Du Pont, Wilmington, Del.). Preliminary trials on three birds showed little or no absorption of ^{14}C FeCN (i.e., essentially no ^{14}C in plasma) and high recovery in excreta (85% ± 12%). Immediately after gavaging, we pushed two artificial fruits into the esophagus, approximately midway between the rictus and proventriculus. Waxwings often store fruits in this region for transfer to the gizzard once the first fruits of a feeding bout have entered the duodenum (Levey and Duke 1992). Waxwings typically eat every 5–15 min and thereby maintain a full gut (Witmer 1994; D. J. Levey, unpublished data). We were fearful that our handling would interrupt this pattern. By placing fruits in the esophagus, we could assure that the radiolabeled solution would be quickly and consistently followed in the gut by another meal.

If birds did not resume eating within 30 min of intubation or did not eat and defecate regularly for the next 2 h, we discontinued the trial and repeated it another day. We collected and recorded the time of each defecation for the first 3 h. Then, we collected and pooled defecations at 195, 210, 225, 240, 300, and 360 min. Mass of food in dishes at the beginning of the trial and 3 h afterward were recorded. The difference, corrected for evaporative loss, is reported as intake.

To control for intrinsic diurnal rhythm of gastrointestinal motility, all trials were started approximately 3 h after room lights came on in the morning. We attempted a complete set of trials with eight birds, but two did not resume eating consistently after intubation and were eliminated from analysis. Several other birds did not eat or defecate regularly in one or more trials. We repeated these trials up to five times and selected the trial with the most temporally consistent pattern of consumption and defecation for use in analyses.

In contrast with many studies, our birds were at no time deprived of food; their passage rates reflect those of unconstrained, freely feeding birds. Also, we stress that the trials were designed to test for rapid modulation of retention time; birds were switched from the maintenance diet to the test diet less than 3 h before retention time was measured. Most other studies on birds that have examined effect of diet on retention time have allowed several days for acclimation to a new diet (Levey and Karasov 1992; Witmer 1994; Karasov et al. 1996; but see Afik and Karasov 1995). Acute regulation of retention time is biologically significant to frugivorous birds because they often switch rapidly from one fruit species to another; that is, their guts often contain more than one species of fruit (Loiselle 1990).

For counting ^{14}C FeCN, we added 1 mL distilled water to each defecation, vigorously shook, and let the solution stand for approximately 18 h at 2°C to assure equilibrium of ^{14}C FeCN with water. For pooled samples, 5–20 mL water was added, depending on the volume of the sample, and 1-mL subsamples were taken for counting. We added 5 mL Scintiverse II (Fisher Scientific, Pittsburgh) to each 1-mL sample. After shaking, vials were left at 21°C in the dark for approximately 24 h, which reduced chemiluminescence. Disintegrations per minute (dpm) of ^{14}C were recorded by a Beckman 5801 liquid scintillation counter, using a quench curve generated by adding ^{14}C to varying amounts of waxwing feces in water.

Assimilation Efficiency

To test the third prediction of the model, glucose apparent assimilation efficiency was estimated using the inert-indicator ratio method (Afik and Karasov 1995) on eight birds eating three concentrations of artificial fruit: 110 mmol/L, 890 mmol/L, and 1,660 mmol/L. Trials were run as described above, except that birds were intubated with a glucose solution containing 18 kBq ^{3}H polyethylene glycol (PEG; molecular weight = 4,000) and 37 kBq D-[^{14}C (U)]glucose (New England Nuclear Research Products, Boston). Excreta were collected and pooled after 1, 4, and 24 h. Samples were processed as described above, except that we used a dual-isotope program that corrected for spill of ^{14}C into the ^{3}H channel. Assimilation efficiency was calculated as

$$100 - 100[(^{3}\text{H dpm/C dpm})_{\text{food}} \times (\text{C dpm}/H^{3}\text{ dpm})_{\text{excreta}}],$$

where the subscripts identify isotope ratios in the intubation solution (food) and in excreta.

Analysis

Total mean retention time (MRT) is the integrated average time between ingestion and excretion (Warner 1981): $MRT = \sum f_i \times t_i$, where f_i is the fraction of total ^{14}C FeCN excreted at time t_i. We used Spearman-rank coefficients of correlation (r_S) to estimate the significance of monotonic relationships among variables and we used coefficients of determination (r^2) to evaluate the goodness of fit of functional relationships to observed patterns.

The influence of ingestion rate and hexose concentration on mean retention time was examined with path analysis (Sokal and Rohlf 1981). Our goal was to determine whether intake alone could adequately explain mean retention time or whether a model including both intake and hexose concentration was needed. To meet the requirement of linear models, mean retention time and intake were log transformed. Log transformation of both variables is recommended when they are related by a power function (Sokal and Rohlf 1981), which was indeed the case (see "Results"). We compared two structural hypotheses (Fig. 3). The first, hypothesis A, assumes that both intake and hexose concentration have direct effects on mean retention time. Hexose concentration also has an indirect effect on retention time through its effect on intake. Hypothesis B assumes that all variation in retention time is due directly to variation in intake; any influence of hexose concentration is indirect. Path coefficients ($P_{y,x}$) shown in Figure 3 are standardized partial regression coefficients. They represent the magnitude of the direct effect of intake on retention time, holding concentration constant ($P_{MRT,I}$), or of concentration on retention time, holding intake constant ($P_{MRT,C}$). The relative magnitude of path coefficients in hypothesis A will indicate which independent variable, intake or hexose concentration, has more influence on mean retention time. The two hypotheses can be compared by their coefficients of correlation (r), which incorporate both

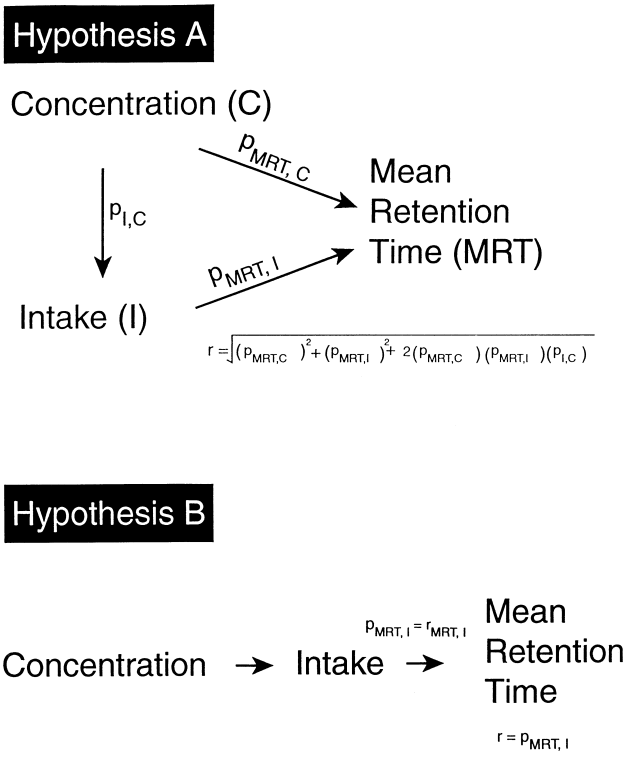

Figure 3. Path diagrams of how intake and hexose concentration in diet affect mean retention time. Hypothesis A posits direct effects on retention time by both hexose concentration and intake and an indirect effect by hexose concentration through intake. Hypothesis B posits a direct effect only of intake; hexose composition of the diet influences retention time only through its effects on intake. Path coefficients, which represent the direct effect of one variable on another while holding the effects of all other variables constant, are shown above the arrows. I = intake, C = sugar concentration, and MRT = mean retention time. Coefficients of correlation (r) describe the overall fit of each model.

direct and indirect effects. We stress that we are not testing one hypothesis against the other. Rather, we ask whether a simple model (hypothesis B) can provide essentially the same explanatory power as a more complex model (hypothesis A).

Degree of kurtosis and skewness of the retention time distributions were estimated using the measures suggested by Bennet and Franklin (1954) to detect mixing in output-time distributions of chemical reactors. These measures are defined as

$$\text{kurtosis} = \sum f_i \times (t_i - \text{MRT})^4/s^4$$

and

$$\text{skewness} = \sum f_i \times (t_i - \text{MRT})^3/s^3,$$

where s^4 and s^3 are the fourth and third powers of the standard deviation of the distribution (i.e., $[\sum f_i \times (t_i - \text{MRT})^2]^2$ and $[\sum f_i \times (t_i - \text{MRT})^2]^{1.5}$, respectively). MRT is total mean retention time and f_i is the fraction of marker excreted at time t_i. The above statistic of kurtosis does not show the positive correlation with the mean (i.e., mean retention time) that other commonly used measurements show (e.g., the fourth moment around the origin; Bennet and Franklin 1954; Zar 1984). Leptokurtic distributions have values of kurtosis greater than three, mesokurtic distributions equal to three, and platykurtic distributions less than three. Symmetrical distributions have a skewness value of zero, distributions that are skewed to the right show positive skewness, and distributions skewed to the left show negative skewness. In all tests we use an α of 0.05 and, unless otherwise noted, report means and their standard errors.

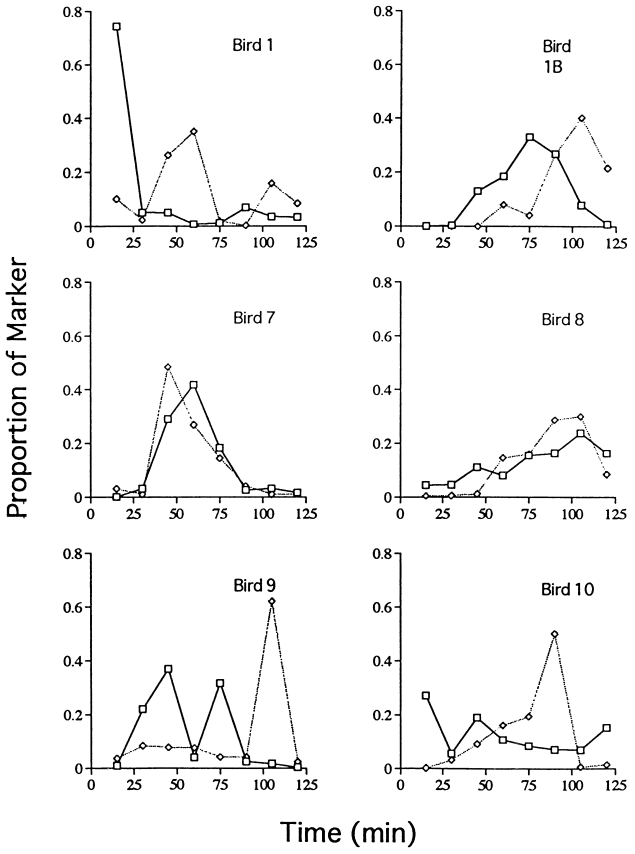

Figure 4. Time of appearance of marker (^{14}C FeCN) in defecations of six waxwings eating 500 mmol/L and 1,660 mmol/L hexose diets (*solid lines* and *dotted lines*, respectively) after intubation at time zero.

Results

Prediction 1: Marker Distribution

Plots of the proportion of ^{14}C dpm excreted versus time were extremely variable in shape, both within and among birds. For illustration, we present and describe curves only for low and high hexose concentrations. Statistics describing the full set of curves are presented below.

In general, differences in curve shape among the five hexose concentrations were not readily apparent by visual examination. Most trials showed a single large peak in excreted ^{14}C (Fig. 4). In no case did the first excretion with ^{14}C contain essentially all the ^{14}C, the telltale sign of a simple plug-flow reactor (Karasov and Cork 1996). Nor was the first pulse typically the largest or even followed by the largest pulse (Fig. 4). Three trials had distinctly bimodal patterns of ^{14}C excretion. None of these curve characteristics fits the expected pattern of an ideal, completely stirred tank reactor in series with a plug-flow reactor (Fig. 2*a*). Forty-two percent of trials had smooth, unimodal curves in which monotonic increases were followed by monotonic decreases in ^{14}C, the expected distribution from a completely stirred tank reactor in series with a plug-flow reactor with much axial mixing (Fig. 2*b*).

Prediction 2: Relationship between Retention Time and Hexose Concentration

Total mean retention time increased significantly from the 110 mmol/L diet to the 1,660 mmol/L diet ($r_s = 0.42$, $P < 0.05$; Fig. 5), which is opposite the predicted relationship.

Prediction 3: Relationship between Assimilation Efficiency and Hexose Concentration

Assimilation efficiencies of glucose were consistently high and did not vary significantly among hexose concentrations (93.8 ± 0.3, 93.5 ± 0.4, and 94.8 ± 0.7 for 110 mmol/L, 890 mmol/L, and 1,660 mmol/L diets, respectively; $F_{2,14} = 2.1$, $P = 0.16$). Again, the model's prediction was not supported.

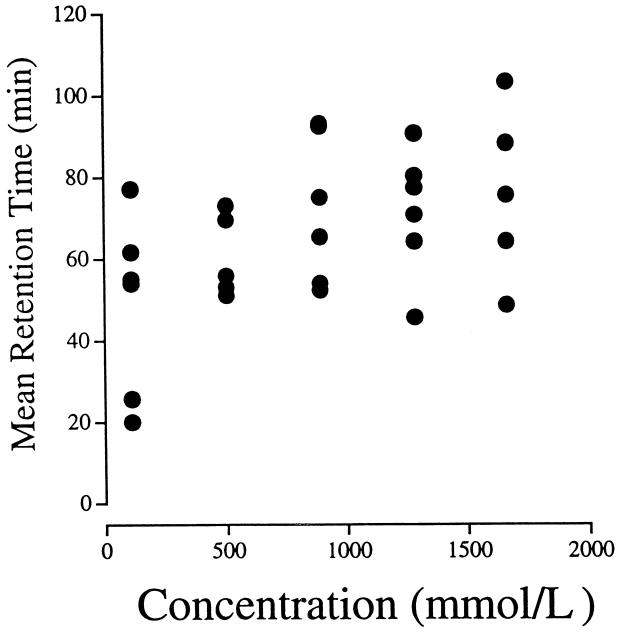

Figure 5. Relationship between gut to cloaca mean retention time and sugar concentration in food. The relationship is significantly positive ($r_s = 0.42$, $P < 0.05$).

Food Intake and Sugar Concentration

Total food consumption increased significantly from 110 mmol/L to 500 mmol/L ($t = 3.4$, $P < 0.01$) and then decreased monotonically with sugar concentration ($r_s = -0.79$, $P < 0.001$; Fig. 6). For concentrations higher than 500 mmol/L, the relationship between food intake and concentration was adequately described by a power function with a slope that did not differ significantly from -1 (slope $\pm$ SE $= -1.05 \pm 0.2$, $t = 0.4$, $P > 0.5$; Fig. 6). This relationship indicates that, for concentrations higher than 500 mmol/L, energy intake was not correlated with sugar concentration ($r_s = 0.04$, $P > 0.5$). Although an ANOVA revealed a significant effect of concentration on sugar intake ($F_{4,25} = 6.3$, $P < 0.005$), this effect ocurred because sugar intake at 110 mmol/L was significantly lower (0.16 ± 0.13 g/h) than sugar intake at all other concentrations (Tukey's multiple comparisons, $P < 0.05$). Sugar intake did not differ significantly among concentrations higher than 500 mmol/L (Tukey's multiple comparisons, $P > 0.1$). Because sugar assimilation efficiency did not differ among sugar concentrations, for concentrations higher than 500 mmol/L, assimilated energy intake rate remained relatively constant.

Direct and Indirect Effects of Intake and Concentration on Mean Retention Time

The overall effect of hexose concentration on total mean retention time was composed of a weak direct effect of hexose concentration ($P_{MRT,C} = -0.36$) and a stronger indirect effect via intake ($P_{MRT,I} \times P_{I,C} = 0.71$; Fig. 7). The direct effect shows that, with the influence of intake held constant, hexose concentration had little effect on mean retention time. Thus, the significant positive association between hexose concentration and mean retention time (see "Results," "Prediction 2") must have been due to hexose concentration's influence on food intake, which in turn was significantly negatively correlated with mean retention time ($r_s = -0.71$, $P < 0.05$). Comparison of the direct effects of food intake and hexose concentration on mean retention time confirms this interpretation; the direct effect of intake exerted a much greater influence on total mean retention time than did the direct effect of hexose concentration ($P_{MRT,I} = -0.97$ vs. $P_{MRT,C} = -0.36$).

Coefficients of correlation were essentially equal for hypotheses A and B ($r = 0.75$ and 0.71, respectively; Fig. 7), indicating that the direct effect of hexose concentration on mean retention time, which was assumed in hypothesis A, had little explanatory value. Thus, effect of hexose concentration on mean retention time appears mostly indirect, via its influence on intake.

The negative relationship between mean retention time and

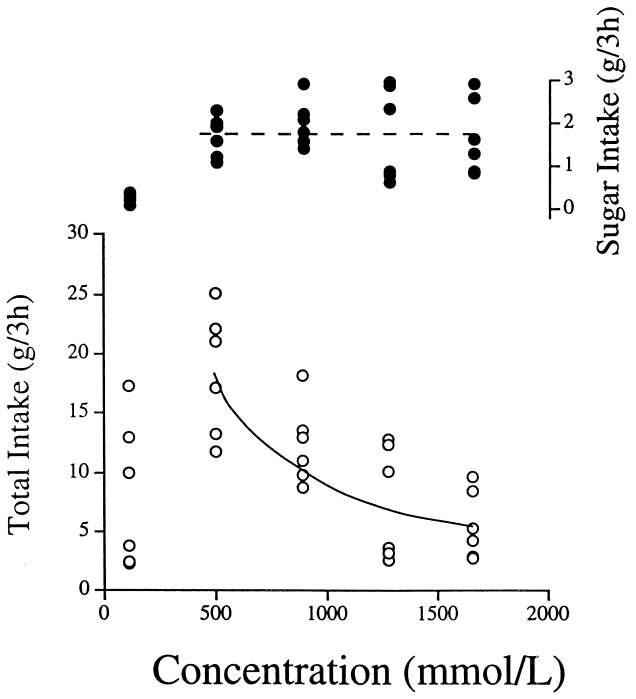

Figure 6. Food intake (*open points*) and sugar intake (*solid points*) as a function of sugar concentration. Note that for concentrations higher than 500 mmol/L food intake decreases with sugar concentration. The curve describing the power relationship between sugar concentration and intake is $Y = 10{,}479X^{-1.05}$, $r^2 = 0.58$. The line in the upper panel represents mean intake for concentrations higher than 500 mmol/L (mean sugar intake $\pm$ SD was 1.76 ± 0.75 13 g/h).

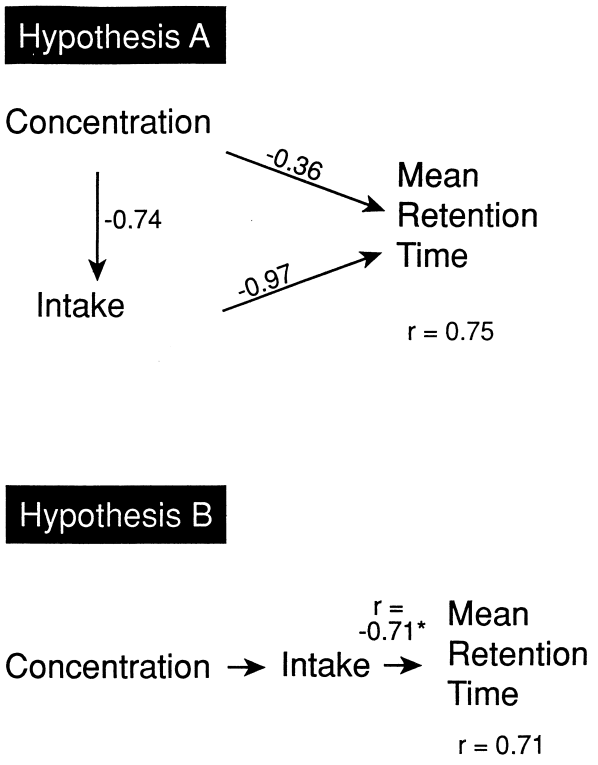

Figure 7. Path coefficients and coefficients of variation for hypotheses shown in Figure 1. Note that direct effects of percent hexose on mean retention time are minimal and that the simpler model, hypothesis B, explains nearly as much variation as hypothesis A. (Asterisk indicates $P < 0.05$.)

intake was well described by a power function ($y = 131x^{-0.24}$, $r^2 = 0.64$; Fig. 8). The exponent of this power function (-0.24 ± 0.07) was significantly smaller in magnitude ($t = 10.3$, $P < 0.01$) than the exponent expected from a simple plug-flow model (exponent = -1; Penry and Jumars 1987). An increase in gut volume at high intake could account for this low exponent.

The product of glucose assimilation efficiency and mass of glucose consumed was remarkably constant in waxwings across a 3.3-fold range of glucose concentration (500 mmol/L – 1,660 mmol/L; $F_{3,15} = 0.79$, $P = 0.35$; Fig. 9). Because assimilation efficiency stayed relatively constant, variation in assimilated glucose was directly determined by variation in mass consumed.

Mixing and Mean Retention Time

Degree of kurtosis was negatively related to mean retention time ($r_s = -0.52$, $P < 0.01$), which suggests an increase in mixing along the gut with increased retention time. All meals that were processed quickly (MRT < 60 min) had kurtosis values greater than 3.0 and therefore had leptokurtic (tall and skinny) distributions of ^{14}C excretion (Fig. 10). Most meals with mean retention times between 60 and 80 min had kurtosis values close to zero, indicating mesokurtic (bell-shaped) distributions. Most meals with mean retention time greater than 80 min had kurtosis values less than 3.0, indicating platykurtic (flat-topped) distributions.

Degree of skewness was also negatively correlated with mean retention time ($r_s = -0.85$, $P < 0.001$; Fig. 11). Eighty-two percent of trials had skewness greater than zero, indicating distributions skewed to the right. Only two trials had essentially symmetrical distributions (skewness = 0), and only one trial had a distribution strongly skewed to the left (skewness < 0).

Discussion

Our results do not support predictions of the model of frugivore gut function proposed by Martínez del Rio and Karasov (1990). We feel confident that we did not make gross errors in measurements since our estimates of retention time and assimilation efficiency are similar to those reported elsewhere for captive cedar waxwings feeding on hexose-rich diets (Witmer 1994 and references therein). The model's poor fit to our data is likely due to invalid assumptions. In this discussion, we critically review the general assumptions of Martínez del Rio and Karasov's (1990) model. Next, we modify some of the as-

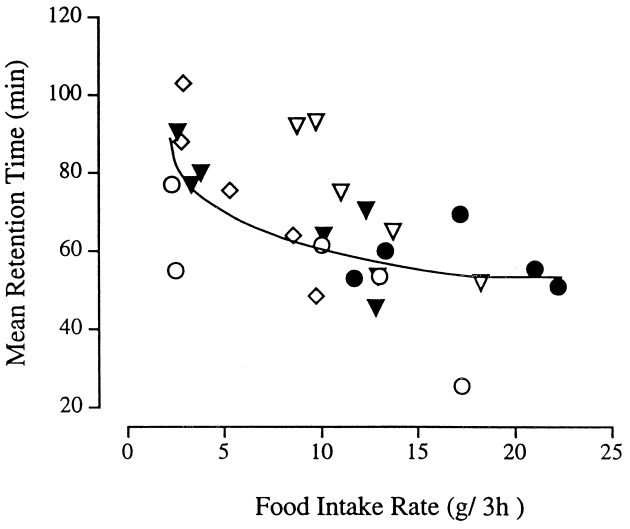

Figure 8. Relationship between total mean retention time and food intake. The power function was fitted with a nonlinear least squares routine (Gauss-Newton). Its exponent (-0.24 ± 0.07) is significantly lower than expected from a simple plug-flow reactor, which suggests an increase in gut volume at high levels of intake (see text). Sugar concentration in food is represented by different symbols (*open circles*, 110 mmol/L; *solid circles*, 500 mmol/L; *open triangles*, 890 mmol/L; *solid triangles*, 1,280 mmol/L; *diamonds*, 1,660 mmol/L).

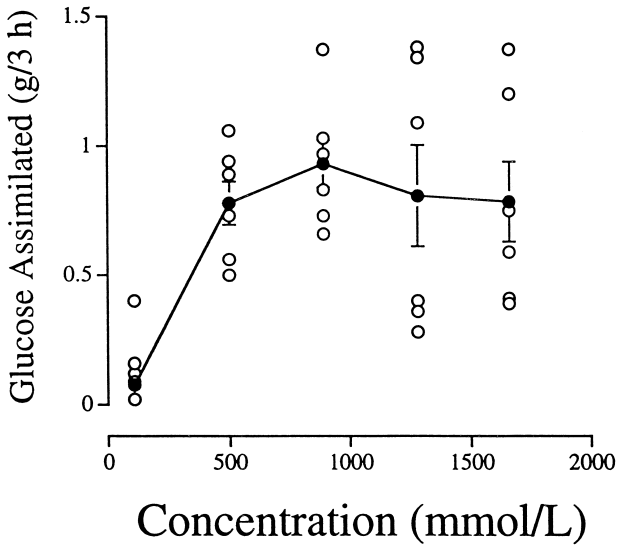

Figure 9. Grams of glucose assimilated by waxwings feeding for 3 h on five diets differing in hexose concentration. Glucose assimilation was calculated by multiplying grams of glucose consumed by average assimilation efficiency of glucose for the three diets tested (110 mmol/L, 890 mmol/L, and 1,660 mmol/L hexose; see text). Bars are standard errors.

sumptions to generate three alternative models of gut function in cedar waxwings. Then, we evaluate these models and provide guidelines for how to test the one that we believe is most consistent with our data and with current understanding of gastrointestinal function in fruit-eating birds. The two final sections of this discussion address the concept of digestive constraints to intake in fruit-eating birds and the relationship between our results and known mechanisms of retention-time modulation in birds.

General Assumptions

The predictions we tested assume that two factors, cost of acquiring food and gut volume, are constant or independent of hexose concentration (Martínez del Rio and Karasov 1990). Cost of food acquisition was certainly constant because food was always presented ad lib. in the same trays. Changes in gut volume are usually assumed to require acclimation periods of weeks to months; our birds had only several hours to acclimate to new diets. Nonetheless, the lower-than-expected exponent of the relationship of mean retention time and food intake suggests that waxwings increased gut volume at high intakes or kept less than completely full guts at low intakes. Observations by Levey and Duke (1992) favor the former explanation, that the wall of the gastrointestinal tract is stretched when full, thereby increasing luminal volume. Indeed, the esophagus of waxwings can expand to store fruit when more is eaten than

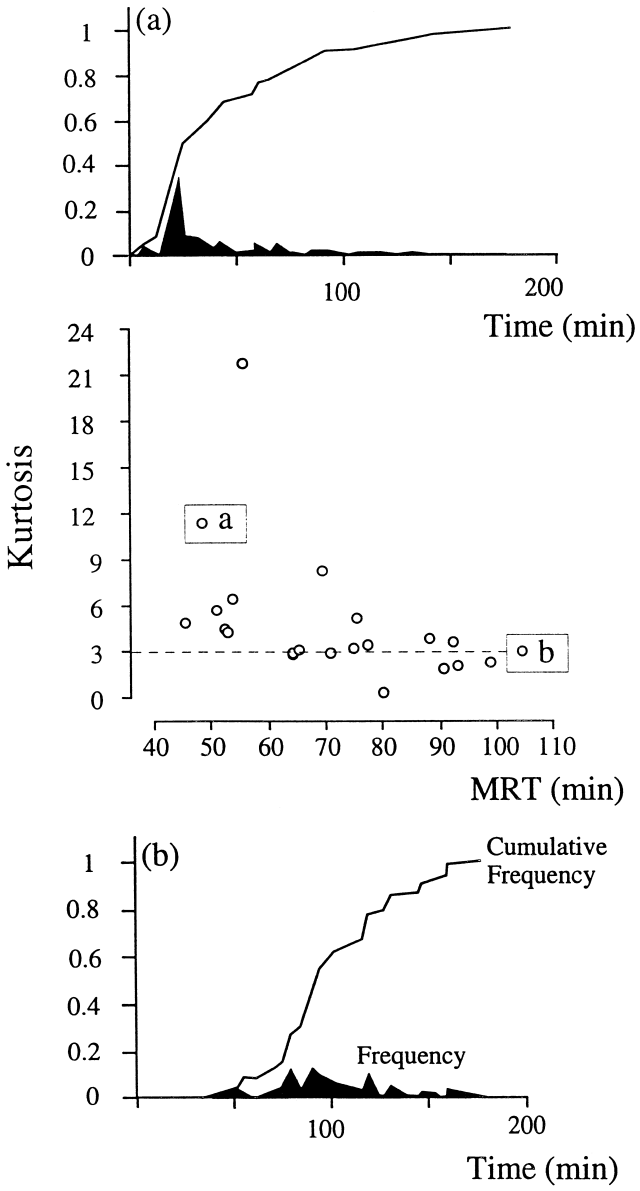

Figure 10. Central panel shows the relationship between degree of kurtosis and total mean retention time. Marker excretion distributions with a kurtosis index higher than three are leptokurtic, those with values equal to three are mesokurtic, and those with values lower than three are platykurtic. The significant negative relationship between kurtosis and mean retention time suggests increased mixing along the gut with increased retention time. To illustrate the effect of mean retention time on the shape of frequency excretion distributions, we also show excretion distribution (*shaded*) and cumulative retention time distribution curves for two birds with low (*upper panel*; point *a* in *central panel*) and high (*lower panel*; point *b* in *central panel*) mean retention times.

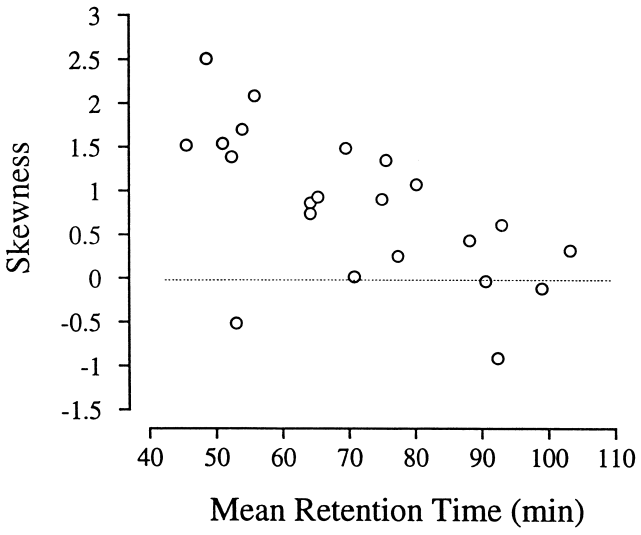

Figure 11. Negative relationship between skewness and total mean retention time. Positive skewness values signify marker excretion distributions that are skewed to the right, negative skewness values signify distributions skewed to the left, and values close to zero signify symmetrical distributions.

the gizzard can immediately process (Levey and Duke 1992). Likewise, an experiment by Levey and Grajal (1991) discounts the latter explanation; waxwings appear to maintain full guts (but see Witmer 1998).

Changes in gut volume critically complicate the most common models of gut function, which assume a fixed volume and can therefore equate flow through the gut to the ratio of gut volume to mean retention time (e.g., Martínez del Rio and Karasov 1990). If volume is not fixed, then the relationship between optimal retention time and optimal intake (analogous to flow) is not easily determined; it depends on the functional relationship between intake rate and gut volume. Note that, in our study, mean retention time decreased as a power function of food intake. The exponent of this function (-0.24) suggests that gut volume increased as a power function of food intake (i.e., gut volume $\propto$(food intake)$^{0.76}$).

Martínez del Rio and Karasov's (1990) model also assumes that most hexose transport occurs as a result of passive processes. Thus, absorption kinetics increase linearly with sugar concentration in food. Achieving constant assimilation efficiencies under these conditions requires that mean intestinal retention time remains constant with sugar concentration in food (P. A. Jumars and C. Martínez del Rio, unpublished data). Thus, the model dictates that food intake rate remains constant as sugar concentration changes. Jumars and Martínez del Rio (P. A. Jumars and C. Martínez del Rio, unpublished data) also demonstrate that, under linear first-order kinetics and constant assimilation efficiencies, sugar assimilation rates should increase linearly with sugar concentration in food. Yet, as Figure 9 and previous results suggest, sugar intake rates in nectar and fruit-eating birds appear to remain constant over a wide range of sugar concentrations in food (Witmer 1994; Karasov and Cork 1996; Downs 1997; López-Calleja et al. 1997). Thus, a simple model with first-order absorption kinetics and constant assimilation efficiency appears to be a poor candidate to describe gut function in fruit- and nectar-feeding birds.

Finally, it is necessary to consider the model's design criterion, which may be incorrect. Like other models of optimal digestion (e.g., Penry and Jumars 1987), the model we tested is based on supply side optimization (Dade et al. 1990), namely, the premise that the evolutionary purpose of gut function is to maximize net rate of energy delivery. This premise explains, for example, why low assimilation efficiencies and rapid gut transit of hexose-rich diets were predicted: because the rate of passive absorption is high at high sugar concentrations and decelerates as hexoses are absorbed and their luminal concentrations drop, the highest rates of hexose absorption can be maintained only by quickly replacing gut contents after the initial, rapid phase of absorption has occurred (Fig. 1). Under this scenario, maximization of digestive efficiency is unimportant, assuming that food is readily available. Yet our birds displayed statistically equal and very high assimilation efficiencies on all diets. Indeed, the assimilation of glucose was almost complete (>93%). This result seems to be prevalent in both nectar- and fruit-eating birds. Karasov and Cork (1996), López-Calleja et al. (1997), and McWilliams and Karasov (1998) found a similar pattern and reached the same conclusion with rainbow lorikeets, hummingbirds, and yellow-rumped warblers, respectively.

Alternative Models of Integrated Gut Function in Cedar Waxwings

Models of gut function in cedar waxwings must be compatible with two observations: (1) food intake decreases with increased sugar concentration (at least for high sugar concentrations) and (2) assimilation efficiency of glucose is high (>90%) and independent of sugar concentration in food. Note that these two observations have been found in several other studies of fruit- and nectar-feeding birds (Witmer 1994; Karasov and Cork 1996; Downs 1997; López-Calleja et al. 1997). Thus, the simple model of Martínez del Rio and Karasov (1990) is an implausible description of gut function in waxwings.

Alternative Model 1: Carrier-Mediated Uptake. Another possible model of gut function in frugivores is based on the assumption that sugar absorption is primarily the result of carrier-mediated processes and, hence, saturable. If the concentration of sugar in the intestine of fruit-eating birds is higher than the Michaelis constant of the transport processes (as is probably the case; Martínez del Rio and Karasov 1990), then an optimal digestion

model predicts almost complete assimilation of hexoses and a reciprocal relationship between food intake and sugar concentration in food. An explanation of these predictions is provided in Martínez del Rio and Karasov (1990).

Two reasons suggest that this model is implausible for cedar waxwings. First, passive absorption appears to be high in waxwings and to account for greater than 84% ± 15% of total glucose absorbed (D. J. Levey, unpublished data). Passive absorption of glucose also seems to be high in every other frugivorous bird tested so far (Afik et al. 1997 and references therein). Second, carrier-mediated rates of glucose transport measured in vitro are insufficient to account for glucose assimilation rates observed in vivo. Average assimilation rates measured in waxwings eating food with a sugar concentration higher than 500 mmol/L were approximately 18 times higher (mean ± SD = 0.28 ± 0.12 g/h) than maximal rates of active glucose uptake for the whole intestine measured in vitro (0.016 ± 0.006 g/h; from Levey and Duke 1992).

Alternative Model 2: Osmotic Constraints. A second, more complex scenario, involves two constraints: (1) an upper limit on the concentration of digesta entering the intestine and (2) a low concentration of sugars in digesta entering the cloaca (i.e., intestinal assimilation of sugars must be high). We call a model including these two constituents the osmotic constraint model. There are physiological reasons to support the notion that these constraints are important in explaining gut function of fruit-eating birds. First, a major role of enterocytes in the duodenum is to maintain luminal osmolarity as close to plasma osmolality as possible (Chang and Rao 1994). In many species, the intestine responds to hypertonic chyme by slowing gastric emptying and diluting chyme to isotonicity via passive flux of water (Chang and Rao 1994). Thus, the maximal osmolarity of sugar in chyme in proximal sections of the intestine does not exceed the osmolality of plasma (Powell 1987). Second, high intestinal assimilation of sugars is necessary to prevent osmotic diarrhea. Although the distal intestine of some bird species can probably transport water against a concentration gradient (Skadhauge 1981), the presence of undigested osmolytes, such as sugars, in the colon probably limits water reabsorption and leads to water and solute losses. Supporting this assertion is the observation that birds unable to assimilate the disaccharide sucrose exhibit excreta osmolarities that are very close to those of plasma (Malcarney et al. 1994).

What is the effect of incorporating these two constraints on a simple model of gut function? For concentrations of food higher than the osmolality of plasma, the model's results do not depend on the mechanism by which sugars are transported. Because the concentration of chyme in the proximal intestine is rapidly diluted to isotonicity, the model predicts that intestinal mean retention times should be independent of sugar concentration in food. Intestinal retention time equals the amount of time required to reduce the concentration of digesta from isotonicity to the concentration in feces in a volume of intestine. Mouth to cloaca mean retention times, however, should be proportional to sugar concentration in food, and thus food intake rate should be reciprocally related to sugar concentration. The reason is that the osmotic constraint model assumes that a constant time in the intestine is required to process a fixed quantity of sugar. Thus, a given volume of food with increased sugar contents will take longer to be processed. A corollary is that ingestion rates will decrease as sugar content increases.

Clearly a test of the osmotic constraint model requires the ability to partition mouth to cloaca mean retention time into intestinal retention time and time in crop, gizzard, and proventriculus. Several studies have used retention time distributions to partition total mean retention time into its component parts (Karasov and Cork 1996; Herrera and Martínez del Rio, 1998). This indirect method is problematical because it depends heavily on how digesta flow is modeled (Karasov and Cork 1996). In particular, it assumes that axial mixing does not occur, an assumption that is clearly false in waxwings (Levey and Duke 1992; and see below). The jagged and multipeaked form of the retention time distributions exhibited by cedar waxwings precludes using this method.

The osmotic constraint model differs from previous models in one important aspect. Other reactor models of gut function assume that assimilation efficiency is a response variable that can be modulated so as to increase energy assimilation rate (Martínez del Rio et al. 1994). This assumption may be untenable for animals such as fruit- and nectar-eating birds that feed on food containing small molecular weight nutrients that are osmotically active. These animals must exhibit high assimilation efficiencies or pay the significant physiological costs imposed by osmotic diarrhea.

The limitations imposed by the osmotic constraint model change the focus of optimal digestion models for fruit- and nectar-eating animals. In particular, investigating the role that modulation of intestinal retention time (and hence of assimilation efficiency) plays in maximizing energy assimilation rates should no longer be the primary goal of these models. Rather, optimal digestion models should explore gastrointestinal designs that maximize energy intake rate, given that the maximal concentration in the intestinal lumen should be close to the osmolality of plasma and that assimilation efficiency of small molecular weight nutrients should be close to complete. Examples of questions that can be addressed by these new generation of models are: What is the optimal balance between passive and active processes for sugar absorption? How should hydrolytic versus absorptive processes be distributed along the intestine's length?

Alternative Model 3: Compensatory Feeding. A commonly invoked notion about ingestion rates is "compensatory feeding." This idea is embodied in "Baumgardt" intake versus food qual-

ity relationships (Van Soest 1994; Castle and Wunder 1995), in which ingestion rates vary inversely with food quality. A prevalent explanation for these relationships is that a constant energy requirement must be met (Collins and Morellini 1979; Downs 1997; López-Calleja et al. 1997). In cedar waxwings, the negative power function with a -1 exponent relating food intake with sugar concentration in food can be interpreted as an example of compensatory feeding.

Compensatory feeding is a competing hypothesis to the osmotic constraint model. Because the osmotic constraint model predicts relatively constant sugar intake rates at high concentration, it is difficult to distinguish between these alternative hypotheses. Experiments increasing the energy demands of animals are a possible way to distinguish between them (see López-Calleja et al. 1997). In particular, under high energetic demands and at high concentrations of sugar, one would expect little change in sugar intake rates under the osmotic constraints model but increased intake rates under the compensatory feeding model. Such experiments, however, must increase energy demands under short-term acute conditions to prevent the responses of the gut that often accompany chronic exposure to increased energy expenditures (Diamond and Hammond 1992; McWilliams et al. 1999).

Energy Balance and Digestive Bottlenecks: What Do Models Tell Us?

Cedar waxwings were able to maintain a relatively constant energy intake when fed food with high sugar concentration but not when fed food with low sugar concentration. Witmer (1994) reported a similar result for captive waxwings on artificial diets. His birds were unable to maintain a constant energy intake when feeding on a 377 mmol/L hexose diet but were able to do so when feeding on 727.1 mmol/L and 1,337.7 mmol/L diets. The failure to ingest sufficient energy at low nutrient concentrations has often been interpreted as the result of volumetric constraints (Levey and Grajal 1991). This notion assumes that the gut is unable to accommodate and process the large volumes needed to achieve compensatory feeding. Our data and the osmotic constraint model suggest an alternative view.

Cedar waxwings fed 110 mmol/L hexoses showed lower, not higher, intake rates, suggesting that their gut was not full to maximal capacity. At low sugar concentrations, assimilation of sugars may be constrained not by the volume that can be accommodated in the gut but by the rate of absorption that can be achieved. In animals with a significant passive component to intestinal sugar uptake, assimilation rates are proportional to concentration in the intestinal lumen (P. A. Jumars and C. Martínez del Rio, unpublished manuscript). Even if birds increase ingestion rates to process food of low concentration at a very high rate, assimilation is limited by low luminal sugar concentrations. Furthermore, ingesting food at very high rates entails high foraging costs.

The low rates of volumetric intake exhibited at low sugar concentrations may be evidence of birds accounting for these costs. At higher intake rates, birds could presumably achieve higher sugar assimilation rates. But as suggested by optimization models, the high costs accompanying these rates counterbalance their benefits. If the osmotic constraint model is correct, increased sugar concentration in food adds a significant benefit to foraging birds. The same energy assimilation rate can be achieved at a lower feeding cost.

Wild fruit-eating birds are faced not only with dilute sugar concentration in fruit pulp but also with the presence of seeds (Levey and Grajal 1991). It long has been hypothesized that seed processing can have a large influence on feeding behavior and energy balance in frugivores. However, the specific effects of fruits' seediness on frugivores' food intake and assimilation remain elusive (Levey and Grajal 1991; Murray et al. 1993; Witmer 1998). Because seeds do not lower the concentration of sugars in solution in the intestinal lumen, their constraining effect on energy intake is different from that of low sugar concentration. Seeds can constrain energy intake because they reduce the intestinal volume that can be filled by assimilable digesta. To attain a more complete perspective of the factors that constrain food intake in fruit-eating animals, future theoretical and experimental studies should incorporate the effect of seeds in fruit on digestive processing.

Regulation of Retention Time

In at least two ways, our results are consistent with the conventional view of gut motility and digesta flow in birds. First, mixing of luminal contents was more prevalent at higher concentrations and longer retention times. This response is likely due to antiperistalsis and intestinal refluxes, in which contents of the duodenum are pushed back into the gizzard (Duke 1986) and the contents elsewhere in hindgut are pushed orally (Levey and Duke 1992). Duodenal refluxes have been observed in waxwings eating a 830 mmol/L hexose diet, although they were rare at that concentration (Levey and Duke 1992). Ileal refluxes and rectal antiperistalsis were common in the same birds (Levey and Duke 1992). In domestic poultry, reflux frequency is positively correlated with concentration of duodenal contents (Duke 1986). Second, the aboral propulsion of digesta from gizzard into intestine is inhibited by high concentration of nutrients, resulting in longer retention times (Duke 1986). This response is complex, involving control of gastric emptying via hormonal and neural reflexes (Duke 1986) and probably mechanisms analogous to the ileal brake described for mammals (Spiller et al. 1984). Note that both of these mechanisms for regulation of gut motility focus attention on concentration of luminal contents and away from other factors that, at the level of the whole animal, may have equal explanatory value. For

example, ingestion rate and retention time are tightly correlated in vertebrates, including frugivorous birds (Warner 1981; Levey and Grajal 1991; Witmer 1994). But, because ingestion rate is also correlated with concentration of nutrients in food, researchers have typically embraced the above mechanisms and discussed concentration effects rather than intake effects. By disentangling the effects of intake and hexose concentration on retention time, our results suggest that the traditional emphasis on nutrient concentration may be unnecessarily restrictive; in whole animals, intake can explain more variation in retention time than can concentration.

Slow modulation of retention time over a period of days or weeks may compromise digestive efficiency when a bird switches diets (Levey and Karasov 1992). The osmotic constraint model suggests that compromising digestive efficiency may lead not only to lower energy intake rates but also to osmotic diarrhea. Although it is likely that intestinal retention time did not vary significantly in cedar waxwings as a function of sugar concentration, our results demonstrated rapid regulation of mouth to cloaca retention time in waxwings. Modulation occurred within several hours (see also Afik and Karasov 1995). Because retention time "may be the single most important digestive feature that is modulated" by birds (Karasov 1996, p. 77), the ability to change it rapidly is likely important to a waxwing's ability to consume and efficiently process different food types over a short period of time. Other frugivorous birds are also able to modulate retention time, some over a similarly short time scale (Levey and Karasov 1994; Witmer 1994; Afik and Karasov 1995; Karasov 1996; McWilliams et al. 1999). Thus, we question the validity of Herrera's (1984) hypothesis that frugivorous birds have inherently rapid passage rates. Retention times are greatly and rapidly modulated, which should facilitate a broad diet. In our study, birds changed total mean retention time 1.5-fold and did equally well in terms of total energy assimilated on diets differing 3.3-fold in hexose concentration.

Conclusion

Optimization studies often cycle between the process of model building and the performance of empirical tests that examine how well the model's assumptions and predicted optima agree with relevant data (Seger and Stubblefield 1996). Our study provides an example of this cyclical process. After testing a model with experimental data, we realized that the assumptions of the original model required reexamination. Reexamination led to the incorporation of new, more physiologically realistic, and apparently crucial assumptions into our model. Adding these assumptions leads to a better match between the model's predictions and our data. This better fit does not "validate" the revised model but rather provides a stimulus for further and more stringent testing. We believe that a proper test of the osmotic constraint model should include the following ingredients: (1) examination of the assumption that the osmotic concentration of digesta does not exceed that of plasma; (2) evaluation of the prediction that intestinal retention times remain relatively constant for higher sugar concentration in food but lead to increased mouth to cloaca retention times, which will require devising a method to estimate intestinal retention time directly, rather than from mouth to cloaca retention time distributions; and, finally, (3) distinguishing predictions of the osmotic constraint model from those of the compensatory feeding model. The compensatory feeding hypothesis predicts that increased energy demands will lead to increased intake, whereas the osmotic constraint model predicts invariant effects of energy demand on intake. Thus, manipulating energy demands should be a critical part of testing the osmotic constraint model.

Modeling guts as chemical reactors has been a valuable tool in comparative digestive physiology because it has provided a descriptive framework for studying the relationship between the complex array of interlocking processes that characterize digestion (Penry and Jumars 1987; Dade et al. 1990; Alexander 1994; Martínez del Rio et al. 1994). Although our empirical data clearly falsified the predictions of such a model, we believe that our iterative approach indicates that the framework of chemical reaction theory can lead to the development of a quantitative and predictive theory of the design and performance of animal digestive systems.

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