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The integration of digestion and osmoregulation in the avian gut

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ABSTRACT

We review digestion and osmoregulation in the avian gut, with an emphasis on the ways these different functions might interact to support or constrain each other and the ways they support the functioning of the whole animal in its natural environment. Differences between birds and other vertebrates are highlighted because these differences may make birds excellent models for study and may suggest interesting directions for future research. At a given body size birds, compared with mammals, tend to eat more food but have less small intestine and retain food in their gastrointestinal tract (GIT) for shorter periods of time, despite generally higher mass-specific energy demands. On most foods, however, they are not less efficient at digestion, which begs the question how they compensate. Intestinal tissue-specific rates of enzymatic breakdown of substrates and rates of active transport do not appear higher in birds than in mammals, nor is there a demonstrated difference in the extent to which those rates can be modulated during acclimation to different feeding regimes (e.g. diet, relative intake level). One compensation appears to be more extensive reliance on passive nutrient absorption by the paracellular pathway, because the avian species studied so far exceed the mammalian species by a factor of at least two- to threefold in this regard. Undigested residues reach the hindgut, but there is little evidence that most wild birds recover microbial metabolites of nutritional significance (essential amino acids and vitamins) by re-ingestion of faeces, in contrast to many hindgut fermenting mammals and possibly poultry. In birds, there is some evidence for hindgut capacity to breakdown either microbial protein or protein that escapes the small intestine intact, freeing up essential amino acids, and there is considerable evidence for an amino acid absorptive capacity in the hindgut of both avian and mammalian hindgut fermenters. Birds, unlike mammals, do not excrete hyperosmotic urine (i.e. more than five times plasma osmotic concentration). Urine is mixed with digesta rather than directly eliminated, and so the avian gut plays a relatively more important role in water and salt regulation than in mammals. Responses to dehydration and high- and low-salt loads are reviewed. Intestinal absorption of ingested water is modulated to help achieve water balance in one species studied (a nectar-feeding sunbird), the first demonstration of this in any terrestrial vertebrate. In many wild avian species the size and digestive capacity of the GIT is increased or decreased by as much as 50% in response to nutritional challenges such as hyperphagia, food restriction or fasting. The coincident impacts of these changes on osmoregulatory or immune function of the gut are poorly understood.

Key words: digestion, osmoregulation, nutrient absorption, gastrointestinal tract, microbial fermentation, birds.

CONTENTS

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I. INTRODUCTION

The vertebrate gut has multiple functions in digestion and osmoregulation. Each of these functions is actually the result of numerous processes performed by different cell types and tissues. Even other organisms may be involved: autoenzymatic digestion is performed with enzymes synthesized by the animal itself, but alloenzymatic (fermentative) digestion is performed with the aid of symbiotic microbes. Although great strides have been made studying these components in isolation and down to a molecular level, our focus on vertebrates as models for studying human disease, or for use as food, or for conservation of biodiversity is also sharpened by study of the components and functions in broader, integrative contexts. These contexts include the ways these different functions might interact to support or constrain each other, the ways they support the functioning of the whole animal in its natural environment, and the way(s) that requisite evolutionary changes in support of one function may have served as constraints in the evolution of other functions. For several reasons, birds provide excellent vertebrate models for studying the performance features and integration of many functions of the gastrointestinal tract (GIT). This review of four major processes and functions of the gut in birds (chemical breakdown of food, absorption of monomers, water absorption, microbially mediated nutritional symbioses) is in the spirit of this integration in mechanistic, ecological, and evolutionary contexts. Besides advancing knowledge in mechanistic physiology of vertebrates generally or of birds in particular, the work has bearing also on ecotoxicology and the emerging field of evolutionary physiology.

II. SEVEN FEATURES IMPORTANT FOR UNDERSTANDING THE INTEGRATIVE FUNCTIONING OF THE AVIAN GUT

(1) Birds have relatively high fuel needs

The phrase ''eating like a bird'' wrongly suggests that birds have relatively small appetites, whereas in fact the typical wild bird eats about a third more dry matter each day than does the typical mammal (Nagy, 2001). Most representatives of both of those taxa are endothermic, meaning that they have five to ten times higher endogenous rates of heat production and hence food requirements than do ectotherms, such as most reptiles, amphibians, and fish. One might think that the extra high energy expenditures of birds relate to the relatively high power requirements for flight, compared with running or swimming, but even birds' rate of energy expenditure measured while fasted and resting in thermal neutrality (so-called basal or standard metabolic rate; BMR or SMR) tends to be higher than in mammals, correlated with their higher body temperatures. All these generalities refer, of course, to comparisons at some specified body size, because metabolic rate and food requirements generally scale with body mass raised to approximately the $^{3}/_{4}$ power [breeding birds may be an exception, as recent analyses show that field metabolic rate

(FMR) of incubating birds scales with a much lower exponent of around ½, Piersma *et al.*, 2003; Tinbergen & Williams, 2002].

Mathematical models of optimal digestion derived from chemical reactor theory (Penry & Jumars, 1987) highlight possible consequences or compensations for the relatively high feeding rates of birds, compared with mammals. For example, larger gut size (i.e. greater total surface area and thus absorptive capacity) in birds relative to mammals would allow digesta retention time and digestive efficiency to be maintained at comparable levels despite relatively higher feeding rates. Alternatively, equal gut sizes among birds and mammals would lead to relatively shorter digesta retention times and lower digestive efficiency in birds because of less contact time between digesta and enzymes and transporters (Karasov, 1996). A third alternative, given equal gut sizes, is that higher tissue-specific digestive enzyme levels or nutrient transport activity would allow birds to maintain comparable digestive efficiency even if digesta retention time were shorter. In the following sections we compare each of these features in birds and mammals – relative size of the gut, digesta retention time, and overall digestive efficiency.

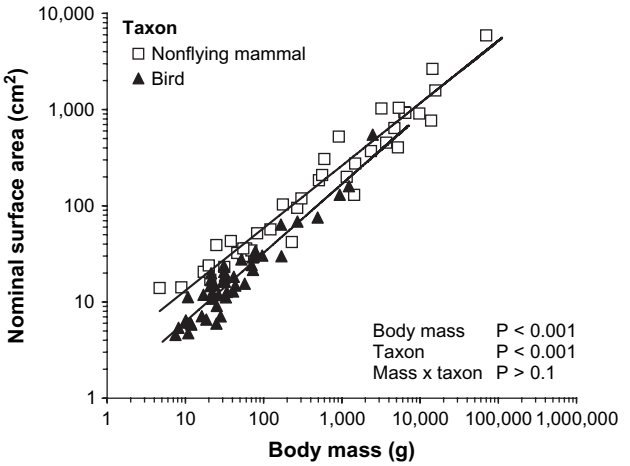

Fig. 1. The small intestinal nominal (smooth bore tube) surface area of birds (filled triangles) tends to be lower than that in nonflying mammals (open squares). No significant difference was found between the slopes of these relationships for these taxa ($F_{1,83} = 2.11$, $N = 46$ species of birds and 41 species of mammals), so the lines were refitted to the common slope of 0.73. Based on calculated proportionality coefficients (intercept at unity, 1.14 for birds and 1.79 for mammals, $F_{1,84} = 47.31$) surface area in birds was approximately 36% lower. Data, analyses and figure modified from Caviedes-Vidal *et al.* (2007).

(2) Birds may have relatively less machinery for extracting fuel from food

The surface area of the gut, where breakdown of substrates and absorption of their monomers occurs, scales with body mass to approximately the $^{3}/_{4}$ power. The allometry of the gut's area has been investigated intensively in mammals (Chivers, 1989; Chivers & Hladik, 1980; Snipes, 1997; Snipes & Kriete, 1991) and to some extent in other vertebrates (Karasov & Hume, 1997; Ricklefs, 1996). The comparative studies show that intestinal surface area is related to the 0.6 – 0.8 power of body mass and that, at a given mass, endothermic mammals have surface areas that exceed those of ectothermic reptiles and fish (Karasov & Hume, 1997). These observations are, of course, what we would expect from an organ that delivers nutrients to fuel metabolic rate. All these studies were based on what has been called nominal surface area, the surface area of the intestine as a smooth tube. It is interesting that the nominal surface areas of birds' small intestines tend to be lower than those of mammals (Fig. 1), as does small intestine length (Caviedes-Vidal *et al.*, 2007; Lavin, 2007). Small intestine volume, a direct function of tube length and area, and consequently the potential mass of digesta carried, is thus relatively smaller in birds. There are differences within birds depending upon diet (e.g. herbivores tend to have the largest small intestinal surface areas and nectarivores the smallest), but the overall differences between birds and mammals held up in a broader analysis of more than 400 species of mammals and birds in which both different diets and phylogeny were taken into account (Lavin *et al.*, 2008). The finding of lower small intestine area in birds may actually be an underestimate of the difference from mammals in absorptive area for fueling metabolic demands. Commonly in mammals, but rarely in small birds, there is additional surface area in the caecum or colon where products from microbial fermentation, such as short chain fatty acids, may be absorbed and account for up to a third of the host's energetic demand (Karasov & Hume, 1997). But, it is well known that the gut surface area of vertebrates is greatly elaborated by finger- and leaf-like extensions called villi and microvilli that increase membrane surface area of individual intestinal cells (Frierson & Foltz, 1992; Karasov & Hume, 1997; Konarzewski & Starck, 2000; Makanya *et al.*, 1997; Moran, 2006; Starck, 2003; Starck & Rahmaan, 2003). Lavin, McWhorter & Karasov (2007) recently showed that birds have significantly greater villus amplification of small intestine surface area than mammals (~1.25-fold more amplification, $F_{1,16} = 7.12$, $P = 0.0096$), with no effect of body size or diet. We could find no published measurements of microvillus amplification in the small intestine of birds. Regardless of potential differences in microvillus amplification, this suggests that birds may have greater mucosal surface area per unit small intestine nominal surface area. However, measurements of nutrient uptake and enzyme activity in the small intestine are not significantly different among birds and mammals when standardized per unit nominal intestine area or mass (Karasov & Hume, 1997). Such standardizations inherently take into account potential differences in villus or microvillus surface area; thus, even if birds have a greater mucosal surface area, a greater catalytic digestive capacity is not necessarily a consequence. So, in those birds studied thus far, increased reaction rates (of mediated transport or enzyme activity) are not a plausible compensatory mechanism for birds with reduced small intestines relative to mammals. Differences

in mucosal surface area may in fact provide one possible mechanistic explanation for higher paracellular nutrient absorption found in small birds: greater villus area per unit intestinal nominal surface area might be associated with more cell junctions across which paracellular transport occurs (see Section V.2). Perhaps there has been natural selection in birds for relatively smaller intestines to minimize body mass and thus lower the power requirements for flight (Dudley & Vermeij, 1992), or perhaps to maximize the volumetric space available for birds' extensive system of air sacs and lungs. Whatever the case, birds appear to be faced with having to satisfy relatively high energy needs with relatively low absorptive surface area. Considering their relatively high food intakes but smaller guts, we might expect relatively shorter digesta retention times in birds.

(3) Birds may have relatively less time for extracting fuel from food

For both autoenzymatic and fermentative digestion, the amount of energy extracted from a meal is a positive function of the rates of breakdown and absorption that occur in digestion chambers and the retention time of food in those chambers (Sibly, 1981). Birds are seemingly at a disadvantage relative to mammals because their digesta retention times are relatively short, according to recent analyses (Lavin, 2007). Digesta retention time at the whole-animal level has been measured in many species by feeding animals indigestible markers and measuring marker excretion from the digestive tract as a function of time since feeding. Most markers are either solutes thought to stay in solution throughout the gut, particulate markers insoluble throughout the gut, or particle markers that become physically or chemically associated with food particles. Amongst the 71 measures of retention time we reviewed (35 in birds, 36 in mammals excluding foregut fermenters), mostly culled from two reviews (Karasov, 1990; Stevens & Hume, 1995), there was no significant difference in the mean retention time of fluid and particle markers ($P = 0.24$, $N = 22$ paired comparisons; Fig. 2). Among both birds and mammals, mean retention time increased with the expected ¼ power of body mass (fitted slope = 0.22, $P > 0.6$ for difference in slope between mammals and birds, Karasov & Hume, 1997). Although retention time can vary according to diet (Robbins, 1993), which was not included as a factor in this analysis, birds had distinctly shorter mean retention times than mammals ($P < 0.001$; Fig. 2). Using a phylogenetically informed analysis that corrected for both diet and body mass, Lavin (2007) recently found that birds had significantly shorter mean retention times of both fluids ($F_{1,63} = 21.82$, $P < 0.001$) and particles ($F_{1,81} = 9.17$, $P = 0.003$) than mammals (on average about 75% shorter), confirming our findings. Diet was not a significant factor in this analysis, and the slopes for body mass *versus* retention time in birds and mammals were also not statistically different (Lavin, 2007). The fact that many mammals exhibit hindgut fermentation, whereas the birds do not, is an important caveat to these analyses. But, this

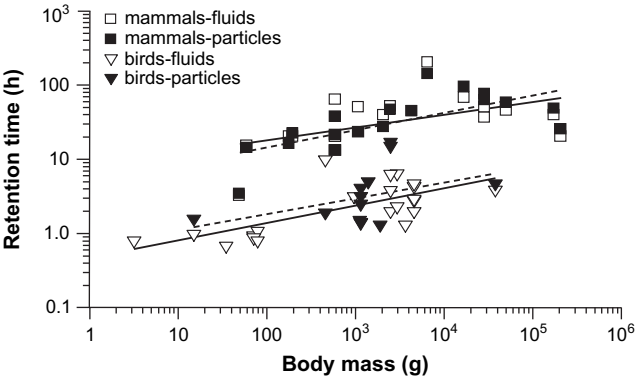

Fig. 2. The retention time of fluid (open points, solid lines) and particulate (filled points, dashed lines) markers in the gastrointestinal system of birds (triangles) is significantly shorter than that in mammals (squares). In this data set, compiled mainly from two reviews (Karasov, 1990; Stevens & Hume, 1995), there was no significant difference between fluid and particle markers ($P = 0.24$ for 22 paired within-species comparisons). Log_{10} retention time increased with log_{10} body mass (pooled slope = 0.22, $P > 0.6$ for difference in slope between birds and mammals), and was significantly higher in mammals compared with birds ($P < 0.001$, $N = 71$ measures).

observation underscores that birds do not compensate for their higher feeding rate with more gut or more digestion time. Does less contact time between digesta and digestive/absorptive surfaces result in relatively low digestive efficiency?

(4) Despite their relative digestive shortcomings, birds are efficient at extracting fuel from some foods

Although they take in relatively more food per day and process it with relatively less intestine and in relatively shorter time, birds do not appear to exhibit dramatically lower digestive efficiency when compared with mammals and ectothermic vertebrates. We are not aware of any systematic comparisons of digestive efficiency among the major vertebrate taxa using the same foods, and so as a first pass at an analysis we compared mean utilization efficiencies reported in taxa-specific reviews with the mean utilization efficiency ± 95% confidence intervals reported for birds in a very large review of hundreds of feeding trial determinations (Bairlein, 1999) (Fig. 3). Utilization efficiencies were calculated as 1-[(faecal + urinary energy output)/(food energy intake)] for reptiles and birds for whom urine and faecal wastes are not separated (see below); for mammals an average small correction for urine loss (Robbins, 1993) was employed when only faecal but not urine energy loss was reported. The comparisons were made by food type, because foods with relatively more material refractory to digestion, such as cell wall material in vegetation, are utilized with lower efficiency than foods with less refractory material such as seeds and invertebrate and vertebrate prey (Karasov, 1990). Utilization efficiencies of

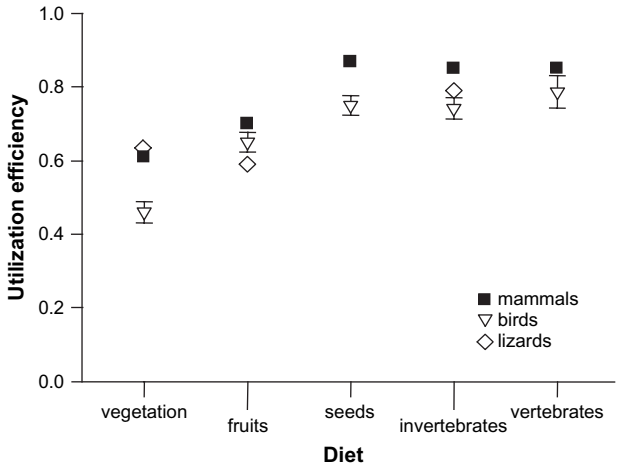

Fig. 3. A comparison of the utilization efficiency (metabolizable energy coefficient) of birds (open triangles), nonruminant mammals (filled squares), and lizards (open diamonds). The data for birds are means ± 95% confidence intervals from Bairlein (1999). Mean utilization efficiencies for lizards are from data in Zimmerman & Tracy (1989) and Marken Lichtenbelt (1992). Mean utilization efficiencies for nonruminant mammals are from Grodzinski and Wunder (1975) and Robbins (1993). Sample sizes (number of feeding trials) are given in the table below.

Taxa	Food type	Number of feeding trials
Birds	vegetation	136
Birds	fruits	147
Birds	seeds	135
Birds	invertebrates	70
Birds	vertebrates	107
Lizards	vegetation	7
Lizards	fruits	4
Lizards	seeds	None
Lizards	invertebrates	11
Lizards	vertebrates	7
Nonruminant mammals	vegetation	21
Nonruminant mammals	fruits	7
Nonruminant mammals	seeds	19
Nonruminant mammals	invertebrates	6
Nonruminant mammals	vertebrates	16

birds consuming invertebrate (insects) and vertebrate prey, and possibly seeds, are similar to those for lizards and mammals (Fig. 3). This finding begs the question whether birds have feature(s) of their auto-enzymatic digestion that compensate for their smaller guts and shorter retention times relative to mammals.

(5) Birds differ from mammals in nutritional provisioning of their gastrointestinal symbionts

Our comparison of utilization efficiencies on wild foods (Fig. 3) suggests that birds may be less efficient than mammals on seeds and vegetation. This lower efficiency of birds on plant matter remains even when species eating similar alfalfa-based diets are compared. Five avian waterfowl species had a mean ± S.E.M. utilization efficiency of 0.37 ± 0.05 (reviewed in Karasov, 1990), significantly lower than the mean (0.57 ± 0.03; $P < 0.005$) for seven rodent species (reviewed in Karasov *et al.*, 1986a) and jackrabbits *Lepus californicus* (Shoemaker, Nagy & Costa, 1976). Klasing (1998) also pointed out that a goose extracts 30% less metabolizable energy from each gram of alfalfa than a rabbit.

Besides relatively shorter digesta retention time, what other differences exist between birds and mammals that might influence microbially mediated fermentation, which is so important for efficient digestion of plant matter? Only one avian species, the hoatzin (*Opisthocomus hoazin*), is known to have true foregut fermentation similar to that found in several groups of mammalian herbivores (Stevens & Hume, 1995). Many of the smallest avian granivores, such as passerines (songbirds), lack an expanded caecum which can act as a fermentation chamber, whereas most small mammalian granivores such as rodents and small marsupials (Hume, 1999; Stevens & Hume, 1995) possess this digestive chamber which can possibly ferment undigested residue that escapes the small intestine. Passerines in the genus *Phytotoma* (''plant cutters'') are among the smallest vertebrate terrestrial herbivores. They weigh only around 45 g and feed almost exclusively on young leaves (Bucher *et al.*, 2003). Plant cutters have a sturdy serrated bill that they use to ''masticate'' leaves which they then process in a short broad intestine that is characterized by unusually high rates of enzymatic hydrolysis (Meynard, López-Calleja & Bozinovic, 1999). Other avian seed- and plant eaters may have caecae, but there are some structural differences from mammalian caecae that we will discuss subsequently.

There are other notable differences from mammals that are relevant to considerations about fermentative digestion. In all birds, the kidney ureters convey urine to the cloaca where it is often refluxed into the colon and caecae (Braun, 2003; Braun & Dantzler, 1997), and the primary nitrogenous excretory product is uric acid rather than urea, as in most mammals. Hence, microbes in the hindgut of birds are provisioned with quite different material to those in the hindguts of mammals, which receive primarily undigested residues of food and urea that diffuses from the blood into the GIT. These differences beg the question whether processes in the hindgut of birds, including those mediated by microbes, are accentuated in birds relative to mammals.

(6) Birds dispose of their absorbed but non-catabolized solutes differently to mammals

In the steady state, the consumption of food is associated with the absorption of numerous ions that must be eliminated. Metabolites from protein deamination, primarily urea in mammals and uric acid (urates) in birds, must also be eliminated, along with biotransformed or untransformed non-nutritive organic compounds such as phytochemicals and toxins. The vast majority of the organic

wastes enter the avian GIT *via* the liver or kidney which both empty into the GIT, whereas in mammals a large proportion may never enter the GIT because urine is conveyed by the ureters to the bladder and thence to the exterior by the urethra. Mammals excrete most ions dissolved in water, possibly at concentrations higher than plasma due to a counter-current multiplication mechanism in the loops of Henlé of kidney nephrons (Braun & Dantzler, 1997). The mammalian kidney is the primary osmoregulatory organ although the urine's sojourn in the bladder may allow for some post-renal modification and the colon's role in dehydration of faeces is certainly important (Braun, 2003; Braun & Dantzler, 1997). By contrast, many birds lack loops of Henlé and cannot excrete hyperosmotic urine, although they certainly regulate urine concentration (e.g. Sabat *et al.*, 2004). Birds excrete ions in a slurry with uric acid that contains a high ratio of solute to water, they lack a bladder, and the intestines of most birds potentially play important roles in osmoregulation, along with salt glands of those species that possess them (Braun, 2003; Braun & Dantzler, 1997). The apparently more prominent role of the avian gut in osmoregulation begs the questions whether some GIT osmoregulatory mechanisms are accentuated in birds relative to mammals, and how the demands of osmoregulatory function(s) constrain or support digestive functions.

(7) Birds naturally deconstruct and reconstruct their gastrointestinal tract

Studies on modulation of the structure and function of the GIT have played a large role in our increased understanding of phenotypic flexibility and the evolution of organismal design (Diamond, 1993; Diamond & Hammond, 1992; Piersma & Drent, 2003), and birds have proved to be excellent models. For example, birds' primary digestive adjustment to chronically increased feeding rate when acclimated to cold temperatures is an enlarged gut (Dykstra & Karasov, 1992; McWilliams, Caviedes-Vidal & Karasov, 1999; McWilliams, Karasov & Caviedes-Vidal, 1996). Birds are not unique in this regard, as similar changes occur in mammals (Karasov & Hume, 1997), but in birds we now know from field captures in summer and winter that these experimentally determined changes actually occur in free-living animals (Battley & Piersma, 2005; Liknes & Swanson, 2003; van Gils *et al.*, 2003). In a somewhat surprising finding, there is a decrease in gut size in many migratory birds (Bauchinger, 2002; Karasov & Pinshow, 1998; Karasov *et al.*, 2004; McWilliams & Karasov, 2001, 2004; Piersma *et al.*, 1999*a*; Piersma & Gill, 1998; Piersma, Gudmundsson & Lilliendahl, 1999b), which perhaps occurs to reduce flight energy expenditure which is dependent on the mass of the body and the size of energy-intensive tissues such as intestine, but may also be the result of catabolism of muscle proteins for energy. In both these situations the mass of the GIT changes by up to 50%. We have some understanding of the nutritional implications of these changes. For example, the gut hypertrophy observed when acclimating to cold effectively increases both gut volume and the total capacity for breakdown and absorption of nutrients, permitting birds to fuel their higher costs of thermoregulation. In migrants, reduced gut size retards their ability to regain body mass rapidly when they stopover to refuel during migration (Karasov & Pinshow, 2000; McWilliams & Karasov, 2001, 2004). We have little understanding of the non-nutritional implications of these changes. Having recognized the dual role of the intestine in digestion and osmoregulation in birds, we can ask how these changes in the gut influence osmoregulatory capabilities. Also, gut-associated lymphatic tissue (GALT) makes up a critical part of the immune system (Albers *et al.*, 2005; Kato & Owen, 1999; Klasing, 2005; Schat & Myers, 1991), but a rarely explored question is whether and how regulation of structure and physiology of the gut is a compromise between digestion and protection. Do the well-documented changes in the GIT resulting from various nutritional states such as hyperphagia or food restriction affect gut immune function (Baker *et al.*, 2004)?

Our short review of seven features important for understanding the interactive functioning of the avian gut identified a number of questions that will guide us subsequently. Might birds have feature(s) of their autoenzymatic- or fermentation-based digestion that compensate for their smaller guts and shorter retention times relative to mammals? Or do they simply have less overall digestive capacity and then operate with a relatively slim margin between that capacity and load (their daily food intake), i.e. do they operate with less digestive "spare capacity"? In Section III, we briefly review the concept of spare digestive capacity and how it has been measured, and describe how studies of disaccharide digestion in nectar-feeding birds within the context of chemical reactor models have provided the best theoretical assessment of this capacity and the only validation of its measurement. In Section IV, we review recent studies that suggest that the avian hindgut appears to exhibit a greater capacity for hydrolysis and absorption than the mammalian hindgut, suggesting a different role in recovering either unabsorbed nutrients from the small intestine or nutritional products of microbial symbionts. In Section V, we review recent studies on birds that reveal a more significant pathway (relative to mammals) for passive absorption of hydrosoluble compounds that could be interpreted to be a compensatory mechanism for birds' smaller guts and shorter retention times. In a similar vein, Section VI relates to the apparently more prominent role of the avian gut in osmoregulation. We conclude by discussing some of the challenging issues in studying the integrative digestive and osmoregulatory function of the gut and highlighting some of the most interesting directions for future research.

III. DIGESTIVE CAPACITY

The primary theme of this section will be the matching of digestive capacity to load. How is digestive capacity best measured? Do birds have less spare capacity than mammals? We begin with a description of how mathematical chemical reactor models of disaccharide digestion in nectar-feeding birds have provided the best theoretical and

empirical assessment of digestive capacity to date. But, because most studies on birds and mammals fall short of this ideal approach, we follow that with a comparative assessment of intestinal hydrolase and transport activities among birds and mammals. Notable differences in the abilities of different taxa to modulate their endogenous intestinal hydrolase and transport activities, potentially explained by phylogenetic or functional mechanisms (or a combination of both), are also outlined.

There is considerable evidence in birds (Karasov, 1996) and mammals (Karasov & Hume, 1997) that digestive features that determine digestive capacity are adjusted in relation to factors such as diet quality and quantity, which determine load. Digestion rate for a particular food or substrate can be greatly increased through changes in digestive organ size, changes in the complement of endogenous enzymes and transport mechanisms for breaking down and absorbing a given substrate, and changes in alimentary tract muscular activity that affect the contact time between substrates and gastrointestinal processes. The relative differences (or ratios) between the absolute maximal digestion rate and the current food intake rate are measures of an animal's "safety margin" (Diamond, 1991) or "reserve capacity" (Diamond & Hammond, 1992) for responding to changes in environmental conditions over different time scales. See McNeill Alexander (1981, 1997) and Diamond (2002) for useful general discussions of safety factors.

These concepts of GIT flexibility and spare capacity are illustrated in Fig. 4. Three points are worth highlighting: (1) at any given time, an animal has some limited spare capacity (called "immediate spare capacity") but this decreases in extent as the GI system reaches its long-term capacity (Hammond *et al.*, 1994); (2) phenotypic flexibility of the GI organs is primarily responsible for an animal's ability to change food intake and diet (i.e. it represents the majority of the "long-term capacity"); however, such phenotypic flexibility requires acclimation time; (3) the maximum rate of metabolizable energy intake achieved after acclimation to energy-intensive conditions (i.e. the plateau value in Fig. 4) may not differ between birds and mammals. For example, according to our analysis of covariance, the near-maximal rate of metabolizable energy intake of birds acclimated to their lowest tolerated temperatures (Karasov, 1990) or engaged in rapid migratory fattening (Kvist & Lindstrom, 2003) are not significantly different ($F_{1,39} = 1.03$, $P > 0.3$) from the near-maximal rates of mammals engaged in lactation (Weiner, 1989, 1992). We reiterate, however, that animals achieve such high rates after digestive changes such as increases in organ size and amounts of enzymes and transporters, and that quantitatively, the survival and fitness benefits of maintaining adequate digestive and absorptive capacity (both immediate and long term) must be balanced against the metabolic cost of maintaining excess capacity.

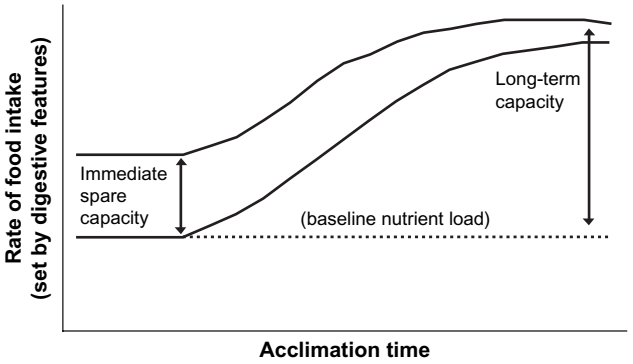

Fig. 4. Immediate spare capacity and long-term capacity (phenotypic flexibility plus immediate spare capacity) for a hypothetical animal exposed to increasing energy demands (e.g. during migration, during cold weather). The solid lower line represents the nutrient load from feeding. Its baseline corresponds to the animal's routine energy demands (e.g. not during migration or at thermoneutral temperatures). The solid upper line represents the capacity of the gut for processing that nutrient load. Capacity on the y axis could be total digestion rate, volumetric intake, nutrient uptake capacity, rate of digestive enzyme activity or some other performance measure of the animal. The x axis is time since the start of an increase in energy demand or change in diet quality. At any given time, an animal can increase its food intake only within the limits set by the level of immediate spare capacity, which decreases as the animal approaches its long-term capacity. When energy and nutrient demands increase, and if the animal has been given time to acclimate fully to these elevated energy demands, then phenotypic flexibility in the digestive system of the animal enables increased energy intake (shown as the increase of the solid lower line above the baseline nutrient load). These changes in digestive capacity are critically important in allowing animals to overcome the challenges associated with changing diet quality or quantity (adapted from Diamond, 1991; Diamond & Hammond, 1992).

(1) Mathematical chemical reactor models used to estimate digestive capacity

Is it possible to estimate digestive capacity at the whole-animal level, and thus maximal digestion and feeding rate, from knowledge of the reaction rates at the tissue level? A number of studies have attempted to do so (e.g. Buddington & Diamond, 1992, 1990; Diamond & Hammond, 1992; Jackson & Diamond, 1995; Lam, O'Connor & Diamond, 2002; O'Connor & Diamond, 1999; Toloza & Diamond, 1992; Toloza, Lam & Diamond, 1991; Weiss, Lee & Diamond, 1998). Most studies estimate capacity by integrating the maximal reaction velocity (V_{max}) of intestinal hydrolases or nutrient transporters along the length of the intestine to yield total hydrolytic or transport capacity for a given substrate. These estimates often exceed nutrient load (daily substrate intake rate) by 100 – 200% (Diamond & Hammond, 1992; Weiss *et al.*, 1998), which is then called the "safety factor" or "spare capacity". If this were correct, it implies that an animal challenged to increase its food intake rate quickly (i.e. within a day or two) could immediately double or triple it. But few critical tests of this idea have been performed, and the few tests that we are familiar with imply a much smaller spare capacity (Karasov & McWilliams, 2005).

The only published study we know of that provides a comprehensive test of this concept was performed with nectar-feeding broad-tailed hummingbirds (*Selasphorus*

platycercus) (McWhorter & Martínez del Rio, 2000). This is an ideal model system because the diet is composed mainly of sucrose, whose catalytic digestion can be characterized by measuring sucrase activity, and because the birds' small intestine accounts for all digestion (they lack a caecum or colon). Sucrase activity was measured with homogenates of tissues collected along the length of the intestine under conditions that saturate the enzyme(s) so that the maximal reaction velocity (V_{max}) could be integrated along length to yield a total hydrolytic capacity. This capacity was about 120% higher than the observed rates of sucrose intake and digestion, implying that the immediate digestive spare capacity was quite high. When faced with an acute metabolic challenge, such as a sudden drop in environmental temperature, the birds should easily compensate by increasing food intake, based on this calculation. But, a behavioural test suggested that this could not be correct, because when the hummingbirds were exposed to low temperature (10 °C) they did not increase their feeding rate to compensate for higher energetic demands, and they lost body mass. The authors pointed out that the common procedure of using the V_{max} over the entire intestine length is physiologically unrealistic, because it implies that transporters at the distal end of the gut are saturated and that the bird would therefore be passing a large amount of unabsorbed sucrose out of the intestine. The loss of such large amounts of osmolyte would elevate water loss, which would certainly be problematic. But also, we know that the birds do not allow much sucrose to escape (they are $\geqslant$ 97% efficient at absorbing sugar), and so the entire approach neglects the reality that the sucrose concentration is progressively lowered as the digesta flows distally along the gut during digestion.

Using a more sophisticated mathematical model of the gut as a plug-flow chemical reactor that included a constraint regarding osmolyte and water loss (Jumars & Martínez del Rio, 1999), McWhorter & Martínez del Rio (2000) calculated a more physiologically realistic digestive capacity that was only 15–35% higher than observed rates of sucrose assimilation. This model used data on intestinal sucrase activity obtained as described above, and calculated maximal catalytic capacity assuming Michaelis-Menten kinetics, but unlike previous attempts it accounted for the physiologically realistic decline in substrate concentration along the length of the intestine (McWhorter & Martínez del Rio, 2000). These authors considered this to be the more accurate estimate of the immediate spare digestive capacity of the broad-tailed hummingbird. Thus, the capacity to digest sucrose seems very closely matched to load, and the bird cannot greatly increase its intake when challenged. In a similar kind of challenge experiment rufous hummingbirds (*Selasphorus rufus*, 3.2 g) switched suddenly to low temperature also could not sufficiently increase their intake and lost body mass (Gass, Romich & Suarez, 1999). A nectar-eating passerine bird, the Palestine sunbird (*Nectarinia osea*), also appears to operate with small digestive spare capacity (estimated as for *S. platycercus*, McWhorter, 2002), and when they were exposed to a relatively sudden drop in ambient temperature (to 5 °C), sunbirds also did not increase their rates of food and energy intake.

In summary, nectar-feeding birds have provided a useful model system for quantitatively testing ideas about digestive capacity because their diets are simple, their autoenzymatic digestion of sugars can be characterized by measuring disaccharidase activity, and their small intestine accounts for all digestion. Incorporation of these features into chemical reactor models with realistic physiological constraints leads to lower estimates of digestive capacity than have generally been described. Furthermore, acute challenge experiments (low temperature in these examples, but reduced feeding time can also be used), in which the birds are forced to rapidly increase sucrose intake and digestion rate, can be used to test quantitatively the prediction of spare capacity. Too few species have been studied using this approach to compare birds and mammals, but most species so studied to date had quite modest immediate spare capacities (range 9 – 50%, Karasov & McWilliams, 2005).

(2) Apparently lower endogenous digestive capacity in birds than mammals

Lacking the ideal data to compare digestive capacity of birds and mammals, we can make a comparative assessment of intestinal hydrolase and transport activities among representatives of the two groups. There do not appear to be fundamental differences between birds and mammals in the primary enzymes and nutrient transporters of the intestinal brush border membrane (Karasov & Hume, 1997). The relative activity of these catalytic agents can be assessed in anaesthetized intact whole animals with *in situ* perfusions, in homogenates of isolated tissue, or in membrane vesicles isolated from intestinal tissue, to name a few of the methods. The largest set of comparable data are available for homogenate- and tissue-based measurements, which are conveniently scaled up to the whole-intestinal level by multiplying activity per unit tissue by total amount of tissue. Thus, for example, sugar and amino acid transport activities have been measured in everted sleeves taken from different regions of the intestine (Karasov & Diamond, 1983) of a large number of avian and mammalian species, and summed uptake capacities over the entire length of the small intestine have been estimated (Karasov, Buddington & Diamond, 1985; Karasov & Diamond, 1988). The measurements are typically made at relevant body temperatures and at substrate concentrations that saturate the sugar and amino acid transporters, and thus are near-maximal rates (V_{max}). It should be mentioned as a caveat here that intestinal tissue of different species of birds (and mammals) reacts differently to tissue handling during the everted sleeve method, and so histological verifications of tissue integrity should be considered when interpreting data (Starck, Karasov & Afik, 2000; Stein & Williams, 2006). In the most recent comparison of such data in birds and mammals (Fig. 7.15 in Karasov & Hume, 1997) there was no significant difference in uptake rate of D-glucose or the amino acid L-proline per nominal cm^2 intestinal tissue, when diet was controlled for (glucose uptake in carnivores tends to be lower than in omnivores; see below). Hence, this type of comparison fails to identify

any compensation in mediated transport for the apparently smaller intestinal nominal surface area of birds relative to mammals (Fig. 1). Below, however, we do suggest that higher passive (paracellular) nutrient absorption might represent such a compensation in birds.

An analogous comparison for intestinal brush border enzyme activity has not been made. A considerable amount of published data exists for hydrolysis rates in intestinal homogenates made under fairly similar conditions (Table 1), many made by us or by colleagues with whom we have collaborated, and so we compared them. We restricted our analysis to omnivores and their brush-border carbohydrases sucrase, maltase and isomaltase and the peptidase aminopeptidase-N. As was the case for measures of transport, hydrolysis rates were typically measured under near substrate-saturating conditions. We compared jejunal or proximal small intestinal hydrolase specific activity (standardized to g protein). We focused on measurements from one region of the intestine, rather than summed activity over the entire intestine, because data on the latter are only available for a much smaller subset of the species we compared. Sucrase, maltase, isomaltase and aminopeptidase-N specific activities [μmol min^{-1} (g protein)$^{-1}$] were not significantly different among mammals and birds ($F_{1,37} = 1.2$, $P = 0.28$; $F_{1,33} = 1.63$, $P = 0.21$; $F_{1,16} = 1.98$, $P = 0.18$; and $F_{1,9} = 2.29$, $P = 0.16$, respectively). Thus, in this analysis also we failed to identify any compensation in autoenzymatic reaction rates for the apparently smaller intestinal surface area of birds relative to mammals. An important caveat to this analysis is that because hydrolytic capacity was compared only in the proximal region of the small intestine, any differences among mammals and birds in the proportion of the GIT with catalytic capacity would of course impact calculations of summed catalytic capacity over the entire intestine.

Table 1. Sources of data on intestinal hydrolysis rates made under similar conditions

Mammalian species	Avian species
Collins *et al.* (1989)	Afik *et al.* (1995)
Deren *et al.* (1967)	Caviedes-Vidal *et al.* (2000)
Goda *et al.* (1983)	Ciminari *et al.* (2005)
Gray (1971)	Karasov & Levey (1990)
Gromova & Gruzdkov (1999)	Malcarney *et al.* (1994)
Hernandez & Martínez del Rio (1992)	Martínez del Rio *et al.* (1995)
Karasov & Levey (1990)	Martínez del Rio *et al.* (1989)
Lam *et al.* (2002)	Martínez del Rio *et al.* (1988)
Lee *et al.* (1998)	Sabat *et al.* (1998)
Lee *et al.* (1983)	Sell *et al.* (1989)
McCarthy *et al.* (1980)	Siddons (1969)
O'Connor & Diamond (1999)	Witmer & Martínez del Rio (2001)
Raul *et al.* (1987)	Zoppi & Shmerling (1969)
Sabat *et al.* (1999)	
Schondube *et al.* (2001)	
Vonk & Western (1984)	
Zoppi & Shmerling (1969)	

Hydrolysis rates were measured using tissue homogenates at optimal pH and temperatures typically appropriate to the vertebrate group (mammals, 37 °C, birds, 40 °C).

Both comparative assessments are admittedly crude because they average across diets and phylogenetic affiliations that can be important sources of variation. For example, aminopeptidase-N activity per unit intestine (length or wet mass) or summed over the entire intestine in hummingbirds was significantly lower than that in other birds ($F_{1,21} = 27.53$, $P < 0.0001$) and bats ($F_{1,21} = 7.82$, $P = 0.011$), consistent with their exceptionally low nitrogen requirements and relatively low intake of insects and hence protein (McWhorter, Powers & Martínez del Rio, 2003*b*). Generally among vertebrates there is a match between enzyme or transporter activity and the predominant dietary substrate (Karasov & Hume, 1997). Animals with carbohydrate-rich diets (nectar, fruit, or seed eaters) tend to have relatively higher levels of carbohydrases (Schondube, Herrera & Martínez del Rio, 2001) and glucose transport activity (Karasov & Diamond, 1988) whereas animals with protein-rich diets (animal consumers) tend to have relatively higher levels of aminopeptidase and amino acid transport activity. But as all of the species we compared were omnivores we feel that our analysis is qualitatively robust because the range of diets used was similar among birds and mammals. Thus, based on the rather similar rates of hydrolysis and mediated transport at the tissue level, the relatively smaller amount of intestinal tissue in birds (Fig. 1) implies a lower endogenous digestive capacity at the whole-animal level.

(3) Adaptive modulation of endogenous digestive capacity compared between birds and mammals

Another possible complication in the comparison of endogenous digestive capabilities is the phenomenon of phenotypic flexibility of both enzymes and transporters in some animals. Dietary modulation of pancreatic and brush border enzymes, and of nutrient transporters, has been demonstrated in many vertebrates (Karasov & Hume, 1997). In the case of brush border enzymes, the overall pattern apparent in most examples of modulation is that activities of sucrase and maltase were increased in animals fed diets higher in carbohydrate, and activities of peptidases were increased in animals fed diets higher in protein (Karasov & Hume, 1997). Analogously, in most examples of modulation of transport by diet composition, D-glucose uptake was increased in animals fed diets higher in carbohydrate, and amino acid transport was increased in animals fed diets higher in protein (Karasov & Hume, 1997). Interestingly, the ability to modulate these catalytic reactions may itself be diet dependent, as omnivores tend to exhibit more, and carnivores relatively less ability to modulate glucose transport (Buddington, Chen & Diamond, 1991; Karasov, 1992). If birds tended to exhibit greater ability than mammals to modulate their digestive enzymes and nutrient transporters, perhaps this could compensate for lower average endogenous digestive capacity.

Available evidence suggests, however, that birds exhibit less, not more, modulation than mammals. In contrast to omnivorous mammals, which may double maximal mediated glucose absorption rate on a high-carbohydrate diet compared to a low- or carbohydrate-free diet (Karasov, 1992), American robins *Turdus migratorius* (Levey & Karasov, 1992), yellow-rumped warblers *Dendroica coronata* (Afik, Darken & Karasov, 1997a), house sparrows *Passer domesticus* (Caviedes-Vidal & Karasov, 1996), and northern bobwhite quail *Colinus virginianus* (Karasov, Afik & Darken, 1996) exhibited little or no modulation of mediated (i.e. active) glucose transport activity *in vitro*. In a later section, however, we do suggest that higher paracellular nutrient absorption in birds is an alternative mechanism to achieve a match between dietary substrate level and absorption rate.

As for modulation of intestinal carbohydrases, recent studies in birds suggest that, here also, they may exhibit less, not more, modulation than mammals. The striking pattern that is emerging is that passeriform and some columbiform birds that do not have functional caecae do not modulate their levels of intestinal carbohydrases, but do modulate intestinal peptidases, in response to dietary substrate concentration. The opposite is true for galliform and anseriform birds which do have functional caecae: intestinal carbohydrases are modulated in response to diet but peptidases are not. This pattern, illustrated in Table 2, suggests several interesting things. First, it suggests that the passeriform and columbiform birds have adequate constitutive enzyme levels in relation to dietary complex carbohydrate load, because their overall efficiency digesting carbohydrate-rich foods such as seeds is relatively high (Fig. 3), and they are not relying on hindgut digestion or fermentation to achieve this. Second, it suggests that non-passerine birds may not rely solely on the small intestinal peptidases for protein digestion. Do the differences between these groups reflect a phylogentic pattern (e.g. modulation of specific activity of aminopeptidase-N as a trait shared by all members of the Superorder Passerimorphae- the taxon above passerines which includes pigeons), or a functional pattern (e.g. birds with functional caecae do not modulate aminopeptidase-N)? In the case of intestinal peptidases, these hypotheses may be complementary: we are not aware of any passerine with a functional caeca and *vice versa*. Ciminari *et al.* (2000) pointed out that permitting small amounts of protein to escape the small intestine would support microbial growth in the caecae of non-passerine birds (see Section IV). By contrast, the small intestine of passerine birds has perhaps been selected (and is able to upregluate its capacity) to extract the maximum available amino acid nitrogen rather than excreting it as waste. Final nutrient extraction in birds with a functional caecum may occur in that organ and, indeed, caecal active sugar and amino acid transport have been described, in some species (i.e. those with large caecal surface area) comprising a significant proportion of the entire intestine's integrated uptake capacity (Obst & Diamond, 1989). Caviedes-Vidal *et al.* (2000) predicted the presence of peptidase activity in the caecum, and it has subsequently been found there in two species of birds (see Section IV). It is interesting that the small intestinal carbohydrase capacity of some passerine birds is much larger than the peptidase capacity (10-fold in house sparrows, Caviedes-Vidal *et al.*, 2000), even though the differences in dietary substrate level are not that great. Perhaps there is a serious risk from excess production of peptidases: rapid degradation of other enzymes. A thorough analysis of the relation between enzyme capacities and nutrient loads, including testing whether low enzyme activity limits reliance on starchy foods, may require additional consideration of the interaction of pancreatic and intestinal enzyme activities with digesta retention and nutrient absorption. For example, relative maltase activity is high in passerine birds but their ability to digest starch is often low (Afik & Karasov, 1995; Feare & McGinnity, 1986; Martínez del Rio *et al.*, 1995), so the limiting step in starch utilization must lie elsewhere. Mathematical models based on chemical reactor theory may be an important tool for integrating the functional capacities of pancreatic and intestinal enzymes with gut size and digesta throughput and nutrient loads, and for estimating both immediate and ultimate digestive spare capacities.

In summary, our review of endogenous digestive capacity and modulation thereof in birds and mammals has considered, but failed to identify, solutions to the riddle of how birds can exhibit digestive efficiencies comparable to those of mammals despite taking in relatively more food per day and processing it with relatively less intestine and in relatively shorter time. Two additional ideas proposed, which will be explored subsequently, are that some birds may rely on digestive mechanisms distal to the small intestine (e.g. in the caecum) to recover nutrients that escape digestion in the small intestine, and that higher paracellular nutrient absorption in birds is an alternative mechanism to achieve a match between dietary substrate level and absorption rate.

IV. THE ROLE OF THE AVIAN HINDGUT IN NUTRITION

As discussed in the previous section, nutrients that escape the small intestine might yet be recovered in the hindgut (Alpers, 1994; Laverty & Skadhauge, 1999). If the hindgut mainly plays this scavenger role, then we must ask what is its added value above and beyond an equivalent mass of small intestine? The answer from the mammalian paradigm comes easily and in several parts. First, although vertebrates lack endogenous cellulase, energy in otherwise indigestible cell wall material becomes available through microbial fermentation in the hindgut in the form of short chain fatty acids (SCFAs) which are absorbed across the mucosa and catabolized for energy by the host. Second, the microbial community synthesizes essential nutrients (vitamins, essential amino acids) which are either absorbed across the mucosa, or reingested in the course of coprophagy (ingestion of faeces) or cecotrophy (ingestion of special caecal faeces, as in rabbits, Hornicke & Bjornhag, 1980; Soave & Brand, 1991). The hindgut microbial community thus can reduce the host's need to forage for energy or essential nutrients. These benefits have been

Table 2. Increment of the specific activity of intestinal carbohydrases and aminopeptidase-N when exposed to an increase of the specific substrate in the diet

	Change in the specific enzyme activity			
	Maltase	sucrase	aminopeptidase-N	Reference
ORDER PASSERIFORMES				
Zonotrichia capensis	no	no	yes	Sabat *et al.* (1998)
Diuca diuca	no	no	yes	Sabat *et al.* (1998)
Sturnus vulgaris	no	not detected	yes	Martínez del Rio *et al.* (1995)
Passer domesticus	no	no	yes	Caviedes-Vidal *et al.* (2000)
Dendroica pinus	yes	yes	yes	Levey *et al.* (1999)
Dendroica coronata	no	no	yes	Afik *et al.* (1995)
ORDER COLUMBIFORMES				
Columba livia	no	no	yes	Ciminari *et al.* (2000, 2005)
ORDER GALLIFORMES				
Gallus gallus	yes	no	not assayed	Siddons (1972)
Gallus gallus (during growth)	yes	yes	not assayed	Biviano *et al.* (1993)
Gallus gallus	yes	no	no	E. Ciminari & E. Caviedes-Vidal (unpublished data)
Meleagris gallipavo	yes	yes	not assayed	Sell *et al.* (1989)
Coturnix coturnix	yes	yes	no	E. Ciminari & E. Caviedes-Vidal (unpublished data)
ORDER ANSERIFORMES				
Branta canadensis	yes	yes	no	Ciminari *et al.* (1998*b*)
Chen caerulescens	yes	yes	no	Ciminari *et al.* (1998*b*)
Anas platyrhynchos	yes	yes	yes	Ciminari *et al.* (2003)

demonstrated empirically for mammals, in which cell wall fermentation in the hindgut has been shown to provide up to as much as a third of maintenance energy needs (e.g. Table 8.3 in Stevens & Hume, 1995) and in which deficiencies of specific essential nutrients, or slower growth rates, are demonstrated when the mammals are made gnotobiotic or restricted from ingesting their faeces (Soave & Brand, 1991; Stevens & Hume, 1995). Considering these demonstrated benefits, it is even possible to argue that the small intestine might have adapted over evolutionary time to release even very digestible materials to the hindgut in order to nurture the important microbial community. For example, fermentable carbohydrates stimulate bacterial growth, which results in enhanced incorporation of nitrogen into bacterial protein (Evenepoel *et al.*, 1999). Analogously, movement of urea-N into the GIT can provide N supplementation in cases where low dietary N levels limit microbial carbohydrate fermentation (Stevens & Hume, 1995). The use of the host's nitrogenous wastes by symbionts is called nitrogen conservation, and nitrogen recycling refers to the situation in which the symbionts use the host's waste nitrogen to manufacture compounds that are then used by the host (Douglas, 1994).

It is unclear whether the hindgut of avian herbivores operates according to this mammalian model because of the paucity of systematic studies on wild birds eating natural diets. We have at times received communications from colleagues about reingestion of faeces by turkeys *Meleagris gallopavo* (G. Duke, personal communication), Gambel's quail *Callipepla gambellii* (E.J. Braun, personal communication), and ostriches *Struthio camelus* (D. Swart & R.I. Mackie, personal communication), and Klasing (1998) claims that preferential consumption of caecotropes (caecal faeces) over rectal faeces, or caecatrophy, is common in several species of Galliformes and ostriches (see also del Hoyo, Elliot & Sargatat, 1992; Mack & Druliner, 2003). It seems widely accepted in the older agricultural production literature that coprophagy is important for meeting the vitamin requirements of poultry (*cf.* Coates, Ford & Harrison, 1968; Klasing, 1998). For example, Klasing (1998) states that deficiencies for several vitamins may easily be induced in poultry when husbandry conditions prevent coprophagy, but rarely occur when they have access to their faeces (see also Monroe *et al.*, 2003). The plausibility of vitamin nutrition *via* coprophagy is arguably balanced by questions about whether behaviour of domesticated species at high stocking densities is a good model for wild herbivores. Coprophagy has been implicated in the transmission of several diseases in captive commercial and experimental poultry flocks (Barnhart *et al.*, 1999*a*,*b*; Hu & McDougald, 2003; Hyun & Sakaguchi, 1989; Montrose, Shane & Harrington, 1985; Trampel, Smith & Rocke, 2005), and drugs have even been developed to attempt to reduce the spread of parasites by reducing coprophagy (e.g. coccidia, see Folz *et al.*, 1986), but the role of coprophagy in most of these studies is confounded by possible alternative modes of disease transmission such as direct contact of individuals (e.g. cloacal pecking) and/or ingesting contaminated feed or water. Currently, direct quantitative data on coprophagy in wild avian omnivores and herbivores, as exisits for mammals

(e.g. Hirakawa, 2001; Kenagy, Veloso & Bozinovic, 1999; Pei, Wang & Wang, 2002; Sukemori *et al.*, 2003), seems to be lacking in the literature. We agree with Klasing (1998) that the quantitative significance of coprophagy/caecotrophy to the amino acid and vitamin requirements of birds awaits further investigation. Also worth considering is whether birds may rely on alternative pathways for recovering microbially synthesized essential nutrients, which we discuss below. Before doing so, we point out some other possibly special features of avian hindguts.

(1) The caecum is the important site of fermentation in most avian species

Herbivory is best known in three groups of birds: grouse (family Tetraonidae in the order Galliformes), waterfowl (Anseriformes, such as geese, swans and some ducks), and ratites such as the ostrich and emu (*Dromaius novaehollandiae*) (Sedinger, 1997). None of them are foregut fermenters. Because the colon or rectum is short in most avian species, and does not have the sacculation necessary for significant microbial fermentation (Klasing, 1998), it is probably not an important site for fermentation as in mammals. There are a few exceptions to this, such as the ostrich, emu, and the northern screamer (*Chauna chavaria*; an anseriform relative of geese), where fermentation in the colon or rectum may be substantial (Swart, Mackie & Hayes, 1993*a*, *b*). But in most birds it is the caecum that contains the prominent microbial community.

There are diverse forms of caeca in birds and generally the extent to which they are developed is characteristic for each major group of birds (McLelland, 1979). The intestinal type is long and resembles the rest of the intestinal tract histologically, including prominent villi (Planas, Ferrer & Moreto, 1987). The glandular type is also long but contains numerous actively secreting crypts. The lymphoid type, which contains many lymphocytes, and the vestigial type, are much reduced in size and probably do not represent important microbial environments. The many proposed nutritional, immunological, and osmoregulatory roles of avian caeca are summarized in several reviews (Klasing, 1998; Laverty *et al.*, 2006; Laverty & Skadhauge, 1999; McNab, 1973), and we will discuss the latter role subsequently in sections on water absorption. As regards microbes, the predominant organisms are obligately anaerobic bacteria that occur in the lumen at approximately 10^{10}–10^{11} g^{-1} (wet mass) (Mead, 1999). Most studies of microbial activity have been on chickens *Gallus gallus*, in which the caecal bacteria are mainly saccharolytic and there is little evidence of cellulose fermentation (Mead, 1999, although see Savory, 1992). But there is evidence of cellulose fermentation in many wild avian species, although this should not necessarily be taken as evidence that cellulolysis is important to the host (Vispo & Karasov, 1997). The microbial communities of most avian species degrade uric acid, but the ability to degrade protein has been little studied and is possibly low judging by the poor ability of chicken caecal microbes to degrade gelatine (Mead, 1999).

The functioning of the caecum has been studied primarily in Galliformes. In wild galliforms the caeca are evacuated each morning when the rest of the tract is virtually empty (Farner, 1960). The filling of the caecum appears to involve mechanism(s) that selectively retain fluid and small particles (including bacteria). In some birds, fluid (urine) is refluxed by antiperistaltic contractions from the cloaca along the usually short colon and into the caeca. This rinses small particles out of the colonic contents and carries them into the caeca (Bjornhag, 1989). Larger particles are left behind to be excreted. Fenna & Boag (1974) argued that in galliform birds a meshwork of ridges and villi at the opening into the caeca prevents large particles from entering the caeca at all. The caeca apparently retains fragments of digesta with high surface area to volume ratio (which are thus relatively rapidly fermented), relatively high concentrations of nutritive substrates from digesta, sloughed GIT epithelia and secretions, and urine, and excludes for rapid defaecation bulkier indigestible material. Interestingly, retrograde urine flow in chickens is increased in hens fed a low-protein diet compared to those on normal or high-protein diet (Waldenstedt & Bjornhag, 1995). This could lead to N recycling (*sensu* the definition above) and an improvement in the hen's N economy. But, this presupposes a way to recover amino acids from the caecum.

(2) How do birds recover nutrients from their caeca?

In birds, as in mammals, the energy in material reaching the caecal microbial community becomes available through microbial fermentation in the form of short chain fatty acids (SCFAs) which are absorbed across the mucosa and catabolized by the host. However, as discussed above, there is little evidence that most species of birds reingest their faeces to the extent that small mammals do, so how would they recover the essential nutrients produced by microbes (vitamins, essential amino acids)? The same question might be asked for humans and other large mammalian hindgut fermenters that do not reingest their faeces. This is a critical, unanswered question for all these organisms, and a situation in which research on one has the potential to increase knowledge for all.

The answer for water-soluble vitamins, which are absorbed across intestinal epithelia partly through transporters, may be emerging from the most recent studies with mammals (Said, 2004). Studies in mammals have shown that there are measurable levels of many of these vitamins in the lumen of the large intestine/colon, and there is accumulating evidence of vitamin transporters at these sites in hindguts of mammals (Said, 2004). We might suppose that research with avian hindgut will similarly show evidence of vitamin transporters there, but we are not aware of any such studies. Older studies of folic acid requirements in poultry denied access to their faeces suggest hindgut absorption of microbially derived vitamins: chickens with intact intestinal microflora reared on diets low in folic acid showed higher haemoglobin and tissue folic acid

levels than their "germ-free" counterparts (Coates *et al.*, 1968; Miller & Luckey, 1963). Although we have a thorough understanding of the vitamin requirements of poultry on a whole-animal level (Klasing, 1998), the capacities for transport of vitamins in the hindgut of birds, if transporters occur there, in relation to transport capacity in the small intestine, minimum requirements and/or daily inputs remain to be evaluated. The vitamin requirements of wild birds are much less thoroughly understood.

The answer for essential amino acids is complicated. First of all, what are the prospects that microbial proteins, relatively rich in amino acids essential to vertebrate hosts (Kinnear *et al.*, 1979), are degraded in the hindgut to small peptides and free amino acids that can be absorbed? Certainly, whole-animal studies may show evidence of protein digestion in hindgut, but if protein is largely degraded by hindgut microbes and then absorbed largely as ammonia, as is often thought to be the case (Li, Sauer & Caine, 1998), this achieves relatively little benefit in regards to satisfying requirements for essential amino acids. Indeed, if the immediate source of the microbial N was the host's urea, uric acid, or high-protein urate "spheres" (Braun, 2003), then one might ask whether the net effect of this kind of microbial cycling of N is anything more than a futile cycle, at least from the perspective of the host's N economy. Similarly, even if the microbes synthesize nonessential amino acids which the host absorbs (Mortensen & Tindall, 1981), the benefits are not obvious. The uric acid or urea originally derives from waste ammonia in the host's bloodstream – ammonia that can be converted to non-essential amino acids without any microbial assistance.

We know of woefully few studies testing for endogenous enzyme activity in the hindgut of birds and mammals that would release essential amino acids to be absorbed. In one fascinating but apparently rarely cited study (Camara & Prieur, 1984), lysozyme was measured at relatively high levels in the distal, but not proximal, colon of rabbits. This enzyme, which degrades bacterial cell walls, was apparently secreted on a circadian rhythm that matched the rhythm at which soft faeces were produced in the caecum and were destined to be ingested during caecotrophy. We are not aware of any studies testing for lysozyme in the avian hindgut. Two studies in poultry (Barash, Nitsan & Nir, 1993; Lepkovsky *et al.*, 1964) suggested that endogenous proteases in the hindgut liberate microbial protein. Yahagi *et al.* (1996) found a faint signal of mRNA for enteropeptidase in rat colon. This paucity of data has led us recently to test more routinely for peptidase activity in homogenates of avian hindgut. In the domestic chicken (*Gallus gallus*, E. Ciminari & E. Caviedes-Vidal, unpublished data), duck (*Anas platyrhynchos*, Ciminari *et al.*, 2004), arctic goose (*Chen caerulescens*, Ciminari *et al.*, 1999), Canada goose (*Branta canadensis*, Ciminari *et al.*, 1998*a*) (Fig. 5), and quail (*Coturnix coturnix*, E. Ciminari & E. Caviedes-Vidal, unpublished data), aminopeptidase-N activity per mg tissue in the proximal caecum was one-third to one-half of that found in small intestine of the same individuals, and in the goose the summed aminopeptidase activity of the caecum accounted for 10–24% of that in the entire GIT (depending on diet; E. Caviedes-Vidal, unpublished data). In these studies tissues were thoroughly rinsed first to remove adherent microbes, but molecular tests could also be used to confirm that the enzyme activity is indeed endogenous. Another troubling question remains though, which is how could a microbial population be cultivated in a reaction chamber (the caecum) which has simultaneously a considerable protease and peptidase activity? Some spatial separation may occur because caecal aminopeptidase activity is highest in the proximal, or "neck" region of the caecum and lowest in the distal sac-like region. This suggests a regional segregation of microbial fermentative activity from enzymatic hydrolytic activity analogous to that described in the rabbit colon (Camara & Prieur, 1984), but this needs further study.

Besides these kinds of studies on possible microbial breakdown, more of which would be welcome, there are

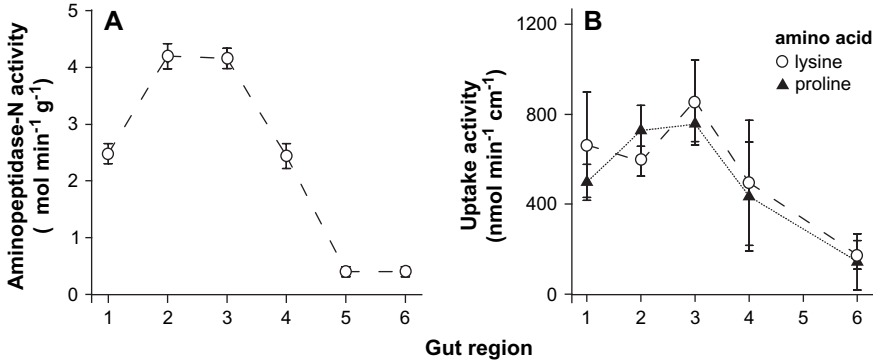

Fig. 5. Variation along the intestinal tract of the Canada goose (*Branta canadensis*) of (A) aminopeptidase-N activity and (B) amino acid uptake activity at the apical membrane, both measured under nearly saturating conditions. The activities were measured in isolated tissue from the small intestine's proximal, medial, and distal regions, and in isolated tissue from the caecum's proximal region near its junction with the intestine to its distal region at the end of the blind sac (1–3 = duodenum, jejunum and ileum, respectively, and 4–6 = caecal tissue moving from the junction with the intestine distally towards the end of the blind sac). The data in A were collected using routine methods, as referenced in Table 1 ($N = 45$ individuals for small intestine $N = 43$ individuals for caeca). The uptake measures in B ($N = 2$ geese) are from Obst and Diamond (1989). All values are means ± S.E.M.

other studies that indicate the presence of free essential amino acids in the lumen of the hindgut. Caecectomized poultry excrete more amino acids in faeces than do controls (Green *et al.*, 1987*a*, *b*; Johns *et al.*, 1986; Kessler, Nguyen & Thomas, 1981; Parsons, 1986), suggesting that dietary amino acids which escape proximal absorption flow into the caeca and that the caeca play some role in absorbing these nutrients (Laverty & Skadhauge, 1999). Based on tests of true digestibility or net protein utilization (which takes into account both digestibility and retention of protein) in poultry, however, most authors conclude that the caeca do not have a significant influence on utilization of dietary protein or amino acid nutrition (Nesheim & Carpenter, 1967; Raharjo & Farrell, 1984; Salter & Coates, 1971; Salter, Coates & Hewitt, 1974; Salter & Fulford, 1974; Sibbald, 1979), although this depends on diet composition (Williams, 1995). Unfortunately, domestic birds have proved to be poor models for the study of caecal function in wild birds, whether because of genetic homogeneity, loss of intestinal microflora diversity, or lack of appropriate dietary preconditioning prior to digestibility trials (Chaplin, 1989; Clench & Mathias, 1995). Regardless, there is evidence of the presence of free amino acids in the hindgut, and indications that essential amino acids are selectively retained: Mortensen (1984) found that the average concentration of seven nonessential amino acids was approximately 1 mmol l^{-1} while that of nine essential amino acids was 0.27 mmol l^{-1}. Are there transporters in the host's epithelium to absorb these amino acids?

Based on numerous studies in birds (Lerner, Sattelmeyer & Rush, 1975; Lind, Munck & Olsen, 1980*a*; Lind *et al.*, 1980*b*; McWilliams, 1999; Moreto *et al.*, 1991; Obst & Diamond, 1989) it seems well established that there is a capacity for carrier-mediated absorption of amino acids in the avian caecum and colon. A number of early studies in mammals indicate the same for adult mammalian hindgut (Ardawi, 1986; Hauge & Krippachne, 1970; James & Smith, 1976; King, Sepulveda & Smith, 1981; Olszewski & Buraczewski, 1978; Robinson, Luisier & Mirkovitch, 1973; Sepulveda & Smith, 1979), although some studies failed to find evidence of active amino acid transport (Binder, 1970; Ilundain & Naftalin, 1981). With the advent of newer molecular methods, researchers are using probes to find amino acid transporters in tissues and cells from the mammalian hindgut (Boll *et al.*, 2002; Ugawa *et al.*, 2001; Utsunomiya, Endou & Kanai, 1996; Yan *et al.*, 1992). Thus, as was the case for vitamin transport in the hindgut, the next step is to determine the capacities for amino acid transport in the hindgut of birds and mammals in relation to the capacity in the small intestine, minimum requirements, or daily inputs. In the proximal caecum of chickens and geese, amino acid uptake rates per unit tissue were half or more of that found in small intestine of the same individuals (Fig. 5B). Indeed, in geese and grouse, caecal amino acid uptake accounts for 6 – 75% of the total uptake capacity of the GIT, depending on species, caecal surface area, and the particular amino acid (McWilliams, 1999; Obst & Diamond, 1989).

What about peptide transport? Chen, Wong & Webb (1999) probed for peptide transporter (PepT1) in adult chicken caecum and found no evidence for it, nor did they find evidence of it in caecum or colon of sheep, dairy cows, or pigs. PepT1 was detected in rat small intestine (duodenum, jejunum, and ileum), but not in the oesophagus, stomach, colon, or rectum (Ogihara *et al.*, 1996). Shen & Smith (2001) found significant PepT1 expression in rat colon during the first week of life, but levels were undetectable shortly thereafter and throughout adulthood. Peptide transport had previously been demonstrated in isolated cells from chick caecum and rectum (Calonge, Ilundáin & Bolufer, 1990), but the ontogenetic study in rats begs the question whether the peptide transport activity in the hindgut of chicks might also disappear during development.

In summary, the once general view that the hindgut did not participate in the digestion and absorption of protein has been undergoing change over the past 10–15 years (Ganapathy, Brandsch & Leibach, 1994). In birds, there is some evidence for a capacity to break down either microbial protein or dietary protein that escapes the small intestine intact, freeing up essential amino acids. There is considerable evidence for an amino acid absorptive capacity in the hindgut of both avian and mammalian hindgut fermenters. Functional interpretation of these capacities awaits more information on the nutrients available to the hindgut epithelium (Obst & Diamond, 1989).

Other kinds of studies attempt to demonstrate more directly recycling of N (*sensu* the above definition). Fuller & Reeds (1998) reviewed studies in which protein was infused into the large intestine of pigs and their N balance was measured. Most of the experiments showed no statistically significant improvement in N balance, although trends in that direction suggested to Fuller and Reeds (1998) that experiments with greater precision might yet demonstrate a significant role of the large intestine to the amino acid economy of pigs. The application of stable isotope methodology offers promise for extending knowledge in this area. In particular, the appearance of labeled lysine in plasma of animals fed [^{15}N]urea or other simple N compounds offers presumptive evidence that this essential amino acid was synthesized by microbes and ultimately absorbed by the host, because lysine does not undergo transamination in the host (Fuller & Reeds, 1998). Similarly, vertebrates cannot incorporate labeled carbon from a carbohydrate source into essential amino acids (except possibly the methyl group of methionine), but microbes can (Torrallardona, Harris & Fuller, 2003*b*). Studies of this sort in both pigs (Torrallardona *et al.*, 2003*b*) and humans (Fuller & Reeds, 1998) have demonstrated absorption of microbially synthesized lysine and other essential amino acids, but we do not know of any such studies in birds. Studies with birds using isotopically labeled compounds have demonstrated N conservation (*sensu* definition above) (Karasawa, 1999; Mortensen & Tindall, 1981; Singer, 2003) but not N recycling that truly improves the avian host's N economy.

Interestingly, comparisons of isotope enrichments in digesta along the digestive tract with those in pig tissues led Torrallardona, Harris & Fuller (2003*a*) to the conclusion that most of the microbially synthesized lysine in pigs was absorbed in the ileum rather than the hindgut. They suggested that there must be a quantitatively important

microbial population in the stomach and small intestine of pigs (coprophagy was prevented in the experiment). This concept challenges our suppositions about major sites of microbial fermentation, and our very human efforts to place animals into neat categories such as "hindgut fermenter". However, the study of the comparative physiology of herbivores prods us to loosen these strictures. Emu, for example, prove that in birds a caecum is not required for extensive fermentative digestion, because their major site of microbial fermentation is apparently the ileum (Herd & Dawson, 1984). Similar examples of this can be found among reptiles (Bjorndal & Bolten, 1990) and fish (Stevens & Hume, 1995). The cross fertilization of concepts and methods among studies on humans, pigs, birds, and the full diversity of vertebrates underscore our point that research on one has the potential to increase knowledge for all.

V. PARACELLULAR ABSORPTION OF WATER-SOLUBLE COMPOUNDS

The products of chemical breakdown of food that are absorbed include many water-soluble nutrients such as monosaccharides, peptides and amino acids from protein, non-protonated short chain fatty acids (SCFAs) from microbial fermentation, as well as a variety of vitamins (e.g. water-soluble B vitamins) and minerals. Because the diffusion of molecules across the phospholipid bilayer membrane of intestinal enterocytes is correlated with their lipid-water partition coefficient (Diamond & Wright, 1969; Smulders & Wright, 1971), this membrane is absorption limiting for these water-soluble molecules. Their transcellular absorption is primarily mediated by membrane-bound transporter proteins that take them up from the gut lumen into the enterocyte across the apical membrane, and hasten their exit from enterocyte to blood across the basolateral membrane. Paracellular absorption involves movement of solutes through the tight junctions adjoining cells (Madara, 1988) by diffusion or by the process of solvent drag (Pappenheimer & Reiss, 1987). The major physical structure defining the permeability properties of the paracellular barrier is the tight junction (Anderson, 2001; Ballard, Hunter & Taylor, 1995). Tight junctions form continuous circumferential intercellular contacts between cells, and the barrier is created where protein particles (fibrils or "strands") in plasma membranes of adjacent cells meet in the paracellular space. Aqueous pores are thought to exist within the paired claudin strands (Tsukita & Furuse, 2000), and this is the putative path for water-soluble compounds. Other processes for absorption occur, such as insorption and persorption (Sass, Dreyer & Seifert, 1990; Volkheimer & Schulz, 1968), but these are probably not nutritionally important in adult vertebrates.

(1) Major features of paracellular absorption

Several studies have measured apparent absorption through the intestinal paracellular space using a series of non-electrolyte hydrosoluble probes that differ in molecular dimensions, such as inert carbohydrates (Chediack *et al.*, 2003; Ghandehari *et al.*, 1997; Hamilton *et al.*, 1987), dextrans (Hill & Shachar-Hill, 1997) or polyethylene glycol (PEG) of varying molecular weights (He *et al.*, 1998; Meehye, 1996; Watson, Rowland & Warhurst, 2001). The organismal approach involves oral gavage or feeding of probes that are non-metabolizable (Caviedes-Vidal & Karasov, 1996; Chediack *et al.*, 2001; Dahlqvist & Gryboski, 1965; Hamilton *et al.*, 1987) and lack affinity for mediated uptake mechanisms (Chediack *et al.*, 2001; Fu *et al.*, 2000; Hamilton *et al.*, 1987). In studies with house sparrows (*Passer domesticus*), carbohydrate probes (L-arabinose, L-rhamnose, perseitol, lactulose; molecular mass 150.1–342.3 Da) were gavaged into nonanaesthetized birds or injected into the pectoralis, and serially measured in plasma (Chediack *et al.*, 2003). Fractional absorption was calculated as $f = [AUC \text{ by gavage}]/[AUC \text{ by injection}]$ where *AUC* is the area under the curve of plasma probe concentration *versus* time. This simple pharmacokinetic method does not require assumptions about pool sizes (e.g. one or two pools) or kinetics (e.g. first order) (Welling, 1986). Sparrows are a good model species because their GIT is very simple, composed of stomach, small intestine (presumably where most absorption occurs), and a short coprodeum. Consistent with predictions, f declined significantly ($P < 0.001$) with probe size by 75% from the smallest to the largest probe, and was significantly ($P < 0.001$) higher in sparrows than in laboratory rats (Fig. 6).

The charge selectivity of the paracellular pathway has been studied by measuring absorption of charged compounds including relatively inert peptides and drugs (Fagerholm *et al.*, 1999; He *et al.*, 1996, 1998; Karlsson *et al.*, 1999; Knipp *et al.*, 1997; Pappenheimer *et al.*, 1994). We compared in house sparrows the fractional absorption of two relatively inert peptides composed of D-amino acids and with different charges, Ser-Lys (233 Da, net charge +) and Ser-Asp (220 Da, net charge −), using the same methodology as described above (Chediack, Caviedes-Vidal & Karasov, 2006). The mean ± S.E.M. fractional absorption was significantly higher (repeated-measures ANOVA on arcsin-transformed values; $F_{1,7} = 6.86$, $P = 0.031$) for the positively charged than negatively charged dipeptide ($f = 0.3 \pm 0.05$ *versus* $f = 0.17 \pm 0.03$). These findings are consistent with cation selectivity of the paracellular route in the absorption of hydrosoluble solutes in the small intestine in birds.

J.R. Pappenheimer and colleagues (Madara & Pappenheimer, 1987; Pappenheimer, 1987; Pappenheimer & Reiss, 1987) claimed that absorption *via* the paracellular pathway could be modulated. Several studies have documented relatively rapid changes in paracellular permeability, apparently triggered by endogenous agents such as cAMP (Perez, Barber & Ponz, 1997), cytokines and leukocytes (Nusrat, Turner & Madara, 2000) and exogenous agents that include dietary constituents such as glucose and amino acids (Madara & Pappenheimer, 1987; Pappenheimer, 1987; Pappenheimer & Reiss, 1987; Pappenheimer & Volpp, 1992; Sadowski & Meddings, 1993), medium chain fatty acids (Lindmark, Kimura & Artusson, 1998) and natural

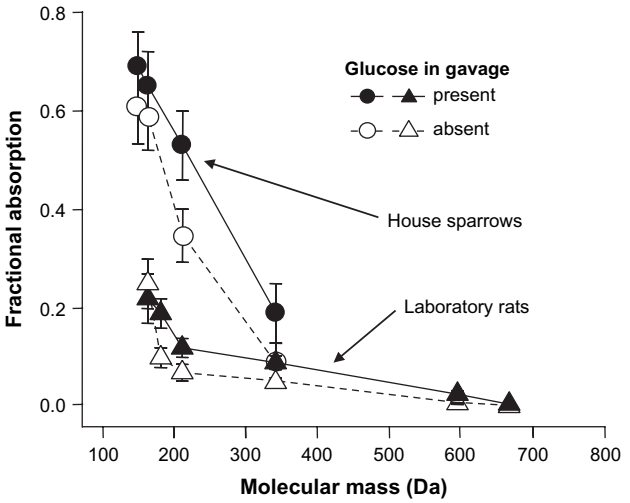

Fig. 6. Paracellular absoption, measured as fractional absorption or bioavailability of different sized metabolically inert carbohydrate probes, in laboratory rats and house sparrows (*Passer domesticus*) in the presence and absence of luminal nutrients. Both species were orally administered (by gavage) solutions containing a mixture of inert probes plus NaCl, with or without 3-O-methyl-D-glucose, which is an actively absorbed but noncatabolized D-glucose analogue. In both studies, and as seen in most other similar studies, fractional absorption declines with increasing size of the inert probe, and absorption increases when coincident with active transport of the D-glucose analogue. Data for sparrows are from Chediack *et al.* (2003), and the data for rats are from Lavin *et al.* (2004) and Lavin (2007). Values ares means $\pm$ S.E.M. $N = 6$.

toxins such as zonula occludens toxin from *Vibrio cholerae* (Wang *et al.*, 2000), capsianoside, a diterpene glucoside from sweet pepper (Shimizu, 1999) and the alkaloid theophylline (Perez *et al.*, 1997). Consistent with the effect of nutrients, fractional absorption of inert carbohydrate probes by house sparrows and laboratory rats was increased by an average of 40% (repeated-measures ANOVA, $P = 0.014$) and 36% ($P < 0.001$), respectively, when the probes were gavaged in the presence of luminal glucose (Fig. 6). In most cases of apparent modulation of paracellular absorption the mechanism(s) are unknown, but may be related to changes in solvent drag because of altered osmotic pressure in the basolateral space and/or cytoskeletal contractions or protein strand alterations that alter the tight junction effective pore size (Madara, Barenberg & Carlson, 1986; Madara & Pappenheimer, 1987; Madara *et al.*, 1988; Pappenheimer, 1987; Pappenheimer & Reiss, 1987). In human jejunum, Na^+-glucose cotransport-dependent regulation of paracellular permeability is associated with phosphorylation of myosin II regulatory light chain (MLC) within the perijunctional actomyosin ring (Berglund *et al.*, 2001).

In summary, a variety of evidence supports the notion of a notable paracellular pathway for absorption of hydrosoluble compounds. Compounds at least up to 342 Da and 0.45 nm radius are absorbed and can be visualized in the paracellular space, and some cation selectivity is apparent. Absorption declines with increasing molecular size, as predicted for a sieve, and is greater in the presence of luminal nutrients and other agents. Knowledge is increasing rapidly regarding the molecular events involved in the modulation of paracellular permeability. A contentious issue has been whether this pathway is physiologically or nutritionally significant.

(2) The paracellular pathway seems especially important in birds

The high fractional absorptions of non-transported, metabolically inert hydrosoluble compounds in granivorous/omnivorous house sparrows (Fig. 6) indicate substantial paracellular absorption. A number of other wild avian species, such as nectarivorous rainbow lorikeets *Trichoglossus haematodus* (Karasov & Cork, 1994), hummingbirds (McWhorter *et al.*, 2006), sunbirds and honeyeaters (Napier *et al.*, 2008), omnivorous yellow-rumped warblers (Afik, McWilliams & Karasov, 1997b) and northern bobwhites (Levey & Cipollini, 1996) achieved nearly complete absorption of ingested L-glucose, the stereoisomer of D-glucose that does not interact with the intestine's glucose transporters and can only be absorbed passively (Chang *et al.*, 2004). The consistency of this finding in birds with diverse diets and taxonomic associations provides strong evidence that, in birds, passive absorption is quite prominent.

The values in birds exceed those in mammals when compounds of similar size are compared (Fig. 7). Below a molecular mass (MM) of 400 Da, analysis of covariance reveals no significant difference in the slope of fractional absorption on MM ($P > 0.1$, mean = - 0.0012), and that the fractional absorption for birds ($N = 21$ measures on six species) significantly exceeds that in mammals (93 measures on seven species) by five times (adjusted least-squares mean $\pm$ S.E.M., respectively, 0.55 $\pm$ 0.03 *versus* 0.11 $\pm$ 0.01, $P <$ 0.001). Of course, many of the studies in each class are on the same species (humans, rats, house sparrows) and thus are not truly independent, so this comparison is crude. Many of the studies with bird species other than sparrows utilized radiolabeled L-glucose, raising the question of whether L-glucose interacts with D-glucose or other transporters or whether the radiolabel becomes disassociated from L-glucose. But tests in birds for mediated L-glucose uptake have been negative (Chang *et al.*, 2004; Karasov & Cork, 1994; Lavin *et al.*, 2007), and checks for radiopurity of labeled L-glucose post absorption have been made (Afik *et al.*, 1997*b*; Caviedes-Vidal & Karasov, 1996; Chang *et al.*, 2004). In all the studies with birds, fractional absorptions were measured by the appearance of probes in plasma (as described above), raising the question of whether that method is biased relative to the urinary recovery method (as described above) that was used in almost every study in mammals. But to our knowledge, no such methodological bias has been recorded in the field of pharmacokinetics. Furthermore, when we used the plasma and urine measurement methods to measure fractional absorption of rhamnose in rats, the fractional absorptions

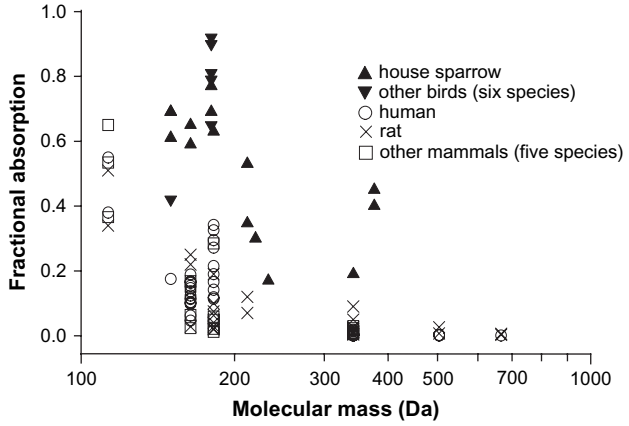

Fig. 7. Fractional absorption of inert probes in birds and mammals. Sources for five avian species are given in the main body of the text, to which data were added for cedar waxwings (*Bombycilla cedrorum*, D.J. Levey, personal communication) and common rock doves (*Columbia livia*, Lavin, 2007). Data for humans were drawn from eight studies cited by Chediack *et al.* (2003), to which data were added from 16 other studies (Bjarnason *et al.*, 1994; Brunetto *et al.*, 1990; Cobden *et al.*, 1985; Erikson & Epsten, 1988; Farhadi *et al.*, 2003; Fleming *et al.*, 1990, 1993; Generoso *et al.*, 2003; Menzies, 1974, 1984; Menzies *et al.*, 1983,1990, 1999; Noone *et al.*, 1986; Saweirs *et al.*, 1985; Wheeler, Menzies & Creamer, 1978). Data for rats were drawn from three studies cited by Chediack *et al.* (2003), to which data were added from four other studies (Martin *et al.*, 2003; Pappenheimer *et al.*, 1994; Sigalet *et al.*, 1996; 2000). Data for other mammalian species include those for mouse (Pappenheimer, 1990), rabbit (Bijlsma *et al.*, 1995; Pappenheimer, 1990), guinea pig (Bijlsma *et al.*, 1995; Delahunty & Hollander, 1987), cat (Bijlsma *et al.*, 1995), dog (Sørensen *et al.*, 1993) and hamster (Delahunty & Hollander, 1987).

did not differ (Lavin, 2007; Lavin, McWhorter & Karasov, 2004; Lavin *et al.*, 2007). Also, there are nagging questions about whether L-glucose (Schwartz, Furne & Levitt, 1995) or L-arabinose and L-rhamnose might have very low affinity for membrane carriers (Bihler, 1969), although no direct evidence for this was found in pigeons (Lavin *et al.*, 2007). Overall, it appears that birds exhibit higher passive absorption than mammals (Caviedes-Vidal *et al.*, 2007; Lavin & Karasov, 2008; Lavin *et al.*, 2007), but this conclusion would be more robust following systematic studies on more species, including additional checks that test probes are truly markers for passive absorption and corrections for diet and phylogeny.

The mechanistic bases for the interspecies differences largely remain to be studied (for an exception to this, see Lavin *et al.*, 2007), but may be the same as those proposed for the effects of modulators on paracellular absorption – different amounts of solvent drag and/or differences in tight junction effective pore size (Madara *et al.*, 1986, 1988; Madara & Pappenheimer, 1987; Pappenheimer, 1987; Pappenheimer & Reiss, 1987).

What is the physiological or nutritional significance of paracellular absorption? This is a pathway for appropriately sized hydrosoluble nutrients such as glucose and amino acids, hydrosoluble drugs and toxins made by humans (e.g. carbamate insecticides, glyphosate herbicide) and naturally occurring toxins in foods (e.g. caffeine, nicotine, some flavonoids). Conceivably, it provides a parallel pathway for mediated absorption of sugars and amino acids and thus could increase the intestine's absorptive capacity. The relatively high paracellular absorption in birds could be interpreted to be a compensatory mechanism for birds' smaller intestinal surface areas and shorter digesta retention times (Caviedes-Vidal *et al.*, 2007). The potential for paracellular absorption of water-soluble nutrients resulting from microbial fermentation in the caeca of birds that do not reingest their faeces remains to be evaluated (see Section IV.2, above), although extensive paracellular absorption seems unlikely given the need to protect against microbial invasion (Klasing, 2005) and regulate salt and water balance across the caecal epithelium (Goldstein & Skadhauge, 2000). The paracellular role in sugar absorption in the small intestine is discussed further below. Whether or not this interpretation is correct, it still seems that high paracellular permeability potentially exposes the animal to higher levels of manmade and natural toxins. This is the rationale for studies of possible enhancers of paracellular drug absorption (Anderbert, Lindmark & Artusson, 1993; Legen & Kristl, 2002; Yamamoto *et al.*, 2001).

Determining whether passive absorption is the primary route of sugar absorption has been the focus of several studies (McWhorter, 2005). Some that argue the point theoretically have focused on issues such as the likely glucose and total osmolyte concentration at the apical surface, possibly influenced by unstirred layers (Ballard *et al.*, 1995; Diamond, 1991; Ferraris *et al.*, 1990; Pappenheimer, 1990, 1993; Pappenheimer & Reiss, 1987). A nonsaturating passive process, as opposed to a saturable mediated process, becomes relatively more important as substrate concentration increases. Another issue raised by Schwartz *et al.* (1995) based on a study in rats was that although high fractional absorption of hydrosoluble probes suggests that there is a prominent nonmediated route for sugar absorption, these probes including L-glucose might be absorbed at a much slower rate than D-glucose but over the entire length of the intestine and thus their fractional absorption could still be fairly high. However, perfused lengths of small intestine in anaesthetized pigeons absorbed passive permeability probe chemicals more rapidly than did perfused lengths in anaethsthetized rats (Lavin *et al.*, 2007).

One elegant experimental approach adopted to resolve this issue has been to compare the rate of absorption of L-glucose (absorbed only passively) with D-glucose or its analogue (absorbed both actively and passively) simultaneously in intact animals, using pharmacokinetic techniques based on measuring levels of these compounds in blood (i.e. integrating uptake measurements over the entire gut through time). In laboratory rats, the absorption rate of nonmetabolizable, actively transported 3-O-methyl D-glucose (3OMD-glucose) apparently exceeded that of L-glucose by about 9:1, implying that most glucose was absorbed actively (Uhing & Kimura, 1995). Similar conclusions have been drawn for dogs (Lane *et al.*, 1999) and humans (Fine *et al.*, 1993), but once again birds appear

to be different. In intact house sparrows, apparent rates of absorption of L-glucose were nearly the same as those for 3OMD-glucose, whether measured under conditions that were relatively saturating or nonsaturating (Fig. 8A-B) for the brush border glucose transporter SGLT1. Under relatively nonsaturating conditions, the least-squares adjusted mean absorption rate for 3OMD-glucose ($1.91 \pm 0.15\%\cdot\text{absorbed}\cdot\text{min}^{-1}$) significantly exceeded that for L-glucose absorption ($1.63 \pm 0.14\%\cdot\text{absorbed}\cdot\ \text{min}^{-1}$) by 17% (repeated-measures ANOVA, $F_{1,62} = 4.01$, $P = 0.049$). Under relatively more saturating conditions, the apparent absorption rates of the two probes did not differ significantly (respectively, 2.59 ± 0.38 *versus* $2.67 \pm 0.42\%\cdot\text{absorbed}\cdot\ \text{min}^{-1}$; $F_{1,45} = 0.1$, $P > 0.7$). Passive absorption apparently accounted for more than 70% of total glucose absorption (Fig. 8C) (Chang & Karasov, 2004). It is important to recognize, however, that 3OMD-glucose is handicapped relative to L-glucose for several reasons. First, the molecular mass of 3OMD-glucose (194.2 Da) is greater than that of L-glucose (180.2 Da), which lowers its diffusion coefficient in water and may decrease its rate of permeation, relative to L-glucose, through the paracellular space which discriminates according to molecular size (Chediack *et al.*, 2003). But, this bias appears to be relatively small (a few per cent, Chang & Karasov, 2004), and can be accounted for in calculations. Also, 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. However, in a recent study in American robins *Turdus migratorius*, McWhorter, Green & Karasov (in press) found that 3OMD-glucose gave estimates of the relative contribution of mediated glucose absorption comparable to those found using radiolabeled D-glucose, when measurements were averaged over the entire absorptive phase (i.e. no apparent bias despite lower affinity and larger molecular mass).

While we think that more and more sensitive measurements will ultimately resolve this issue, we reiterate that we do not believe that the biological significance of passive absorption hinges mainly on whether most glucose is absorbed this way. It seems plausible that paracellular absorption provides a parallel pathway to mediated

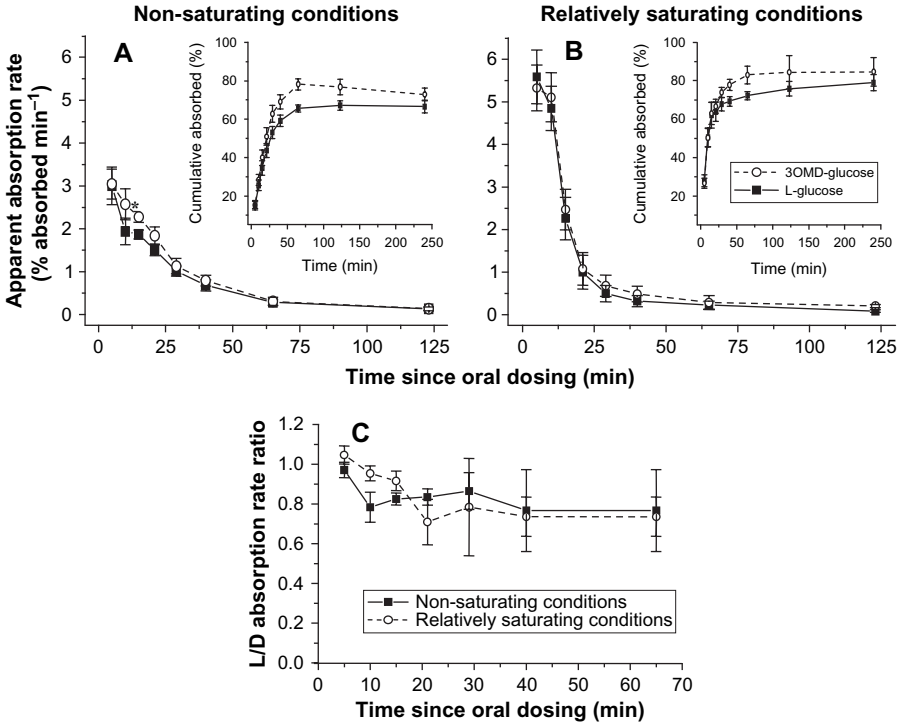

Fig. 8. Results of an experiment to determine the fraction of sugar absorption that is passive in house sparrows *Passer domesticus* (from Chang & Karasov, 2004). A and B show the cumulative absorption (inset plots) and apparent rates of absorption of [^{3}H]3-O-methyl-D-glucose (3OMD-glucose) and [^{14}C] L-glucose (L-glucose) as a function of time since gavage of the probes to house sparrows. The plot and inset in A are for measurements made under relatively nonsaturating conditions (200 mmol l^{-1} mannitol in the gavage solution, $N = 7$ birds), and the plot and inset in B are for measurements made under more saturating conditions (200 mmol l^{-1} 3OMD-glucose replaced mannitol in the gavage solution, $N = 6$). Filled symbols and solid lines represent L-glucose, and open symbols and dashed lines represent 3OMD-glucose. (C) Ratio of apparent absorption rates for L-glucose and 3OMD-glucose. Assuming that absorption of L-glucose is passive whereas the absorption of 3OMD-glucose represents the sum of passive and mediated absorption, the ratio of the apparent absorption rates (L/D) indicates the proportion of 3OMD-glucose absorption that occurs *via* the paracellular pathway. The ratios were calculated from the apparent rates shown in A and B, for measurements made under relatively nonsaturating conditions (filled squares, solid line), and for measurements made under relatively more saturating conditions (open circles, dashed line). Values ares means $\pm$ S.E.M.

absorption of sugars and amino acids at high dietary levels and thus increases the small intestine's absorptive capacity. It also seems plausible that its enhancement in birds relative to mammals partially compensates for typically shorter intestines in representatives of the former group. Even if these interpretations are ignored, it seems a fact that the paracellular pathway provides a route of absorption for hydrosoluble toxins and drugs up to a specific molecular size (Sugano *et al.*, 2003; Tavelin *et al.*, 2003). The occurrence and possible regulation of this route (Anderson & van Itallie, 1995; Ballard *et al.*, 1995) thus has important implications for nutrition and drug design. Furthermore, vulnerability to hydrophilic toxins could be an important ecological driving force, constraining food exploratory behaviour, limiting the breadth of the dietary niche, and selecting for compensatory behaviours such as searching for and ingesting specific substances that inhibit hydrophilic toxin absorption (Diamond, Bishop & Gilardi, 1999).

VI. THE ROLE OF THE AVIAN GUT IN SALT AND WATER REGULATION

We summarized earlier several important features of the avian osmoregulatory system (Section II.6). Here we begin by reviewing recent studies of supply-side water balance regulation in nectar-feeding birds, and then describe the role of the hindgut in regulating water and electrolyte balance.

(1) Supply-side water balance regulation in nectar-feeding birds

To fuel their exceptionally high mass-specific metabolic energy demands, nectar-feeding birds often experience water fluxes closer to those experienced by amphibians and freshwater fish than those of endothermic vertebrates (Beuchat, Calder & Braun, 1990). Hummingbirds and sunbirds may consume 3–6 times their body mass in nectar per day under energetically demanding conditions (McWhorter, Martínez del Rio & Pinshow, 2003*a*; Nicolson & Fleming, 2003). Beuchat *et al.* (1990) hypothesized that when hummingbirds are ingesting large volumes of dilute nectar, perhaps only a small fraction of the water is absorbed in the small intestine, leaving the rest to pass quickly through the intestinal tract. This hypothesis was an attractive explanation for the ability of these birds to process such large volumes of water rapidly, but it presented certain digestive challenges: it requires the rapid absorption of sugars and electrolytes and strict regulation of transepithelial water flux (Beuchat *et al.*, 1990; Skadhauge, 1981). If ingested water is largely absorbed across the intestine, as appears to be the case in most vertebrates (Powell, 1987), nectar-feeding birds would be faced with significant renal challenges for water elimination and glucose and electrolyte recovery when feeding on dilute nectars (although as discussed below, the distal GIT also plays an important role).

McWhorter & Martínez del Rio (1999) used pharmacokinetic techniques to estimate the fractional absorption of ingested water across the GIT of birds (i.e. the proportion that contributes to body water turnover). They tested and rejected the hypothesis of Beuchat *et al.* (1990) in broad-tailed hummingbirds (*Selasphorus platycercus*): approximately 80% of ingested water contributed to the turnover of the body water pool and fractional absorption was not correlated with food or water intake or diet energy density. Hartman Bakken & Sabat (2006) recently confirmed in the Chilean green-backed firecrown (*Sephanoides sephanoides*) that hummingbirds absorb most dietary water (90% for this species). McWhorter *et al.* (2003*a*) examined the relationships among nectar intake, water absorption and water turnover in the Palestine sunbird (*Nectarinia osea*), a member of a taxon considered to be one of the other major radiations of nectar feeding in birds (Nectariniidae). They found that these sunbirds do something very different than the hummingbirds: they modulate water absorption across their intestine. Fractional water absorption decreased asymptotically from 1 (or 100%) when birds were feeding on concentrated sucrose solutions (low water intake) to about 0.36 when they were feeding on dilute solutions (high water intake) (Fig. 9). These results suggest that Palestine sunbirds avoid absorbing, and thus having to eliminate, up to 64% of ingested water when intake rates are high. The volume of water absorbed per mass sucrose assimilated decreased with increasing nectar sucrose concentration, suggesting that sunbirds can regulate transepithelial water flux independently of sugar absorption. To our knowledge this is the first documentation of apparent adaptive regulation of absorption of ingested water across the GIT to the body in a vertebrate.

Modulation of intestinal water absorption requires the rapid absorption of dissolved sugars and efficient extraction of electrolytes and amino acids present at low levels in ingested nectar (Beuchat *et al.*, 1990). It also requires that the permeability of the intestine to transepithelial water flux be regulated. How do sunbirds regulate water flux, while rapidly absorbing osmotically active sugars and electrolytes, while hummingbirds do not? Differences in mechanisms of sugar absorption and mass-specific metabolic rates among hummingbirds and sunbirds may explain the apparent ability of sunbirds to modulate water absorption. The mechanisms of intestinal water absorption in nectar-feeding birds are unknown but are probably facilitated by sugar uptake. Hummingbirds exhibit the highest rate of carrier-mediated glucose uptake measured in a vertebrate (Karasov *et al.*, 1986*b*), and the Na^+/glucose cotransporter (SGLT1) may move significant volumes of water (up to 4.8 l water per mole of glucose: Loo *et al.*, 1996). McWhorter & Martínez del Rio (1999) estimated that the amount of water potentially accompanying mediated glucose absorption in broad-tailed hummingbirds exceeded the water content of the nectar consumed by 1.7– to 5.5-fold, depending on sucrose concentration. McWhorter *et al.* (2003*a*) similarly estimated that the volume of water potentially accompanying mediated glucose absorption in Palestine sunbirds exceeded their water intakes (based simply on known glucose assimilation efficiency, and assuming that all glucose uptake is mediated). This comparison is perplexing because the sunbirds appear to be able to regulate water absorption whereas hummingbirds do not. Perhaps the permeability of sunbirds' intestines to transepithelial water flux increases

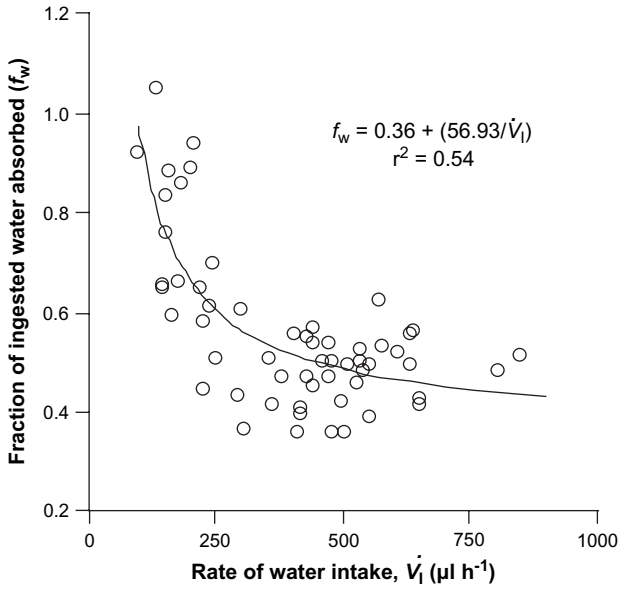

Fig. 9. The fractional absorption of ingested water (f_w) across the gut of Palestine sunbirds *Nectarinia osea* ranged from 0.33 to 1.02 (mean ± S.E.M., 0.59 ± 0.04, $N = 35$) and declined significantly and non-linearly with water intake rate ($F_{1,29} = 40.03$, $P < 0.0001$, $r^2 = 0.54$).

with sugar concentration. For example, sunbirds may have a low capacity for mediated glucose uptake relative to hummingbirds and thus might rely more on passive paracellular absorption of nutrients at high sugar concentrations. Passive absorption is an important route for nutrient absorption in some passerine and psittacine birds (Afik *et al.*, 1997*b*; Caviedes-Vidal & Karasov, 1996; Chediack *et al.*, 2001; Karasov & Cork, 1994), as well as in hummingbirds (McWhorter *et al.*, 2006) and nectar-feeding honeyeaters and sunbirds (Napier *et al.*, 2008), but the mechanisms by which it is regulated are poorly understood. It would be instructive to measure the capacity for mediated glucose uptake in sunbirds and determine whether the relative contributions of passive glucose absorption and epithelial permeability vary with water intake, given constant energy intake, differently to hummingbirds. It is also important to note that the estimates of the capacity for water absorption *via* mediated Na^+-glucose cotransport in nectar-feeding birds described above were based on measurements on the mammalian SGLT1 expressed in the *Xenopus laevis* oocyte made by Loo *et al.* (1996). Their measurements sought to isolate water transport by that cotransporter and represent but one element in a complex membrane system.

The studies described above were done in two species of hummingbird and one species of sunbird. It remains to be seen whether adaptive regulation of dietary water absorption is a general pattern in sunbirds or even all specialized passerine nectarivores and whether the lack thereof is a general pattern in hummingbirds. At present the links between nutrient absorption and the regulation of trans-epithelial water flux in nectar-feeding birds remain a mystery.

(2) The avian hindgut is involved in water and salt regulation

Despite a renal urine-concentrating ability that is relatively lower than in mammals (Braun, 1997), birds are as effective at conserving water and salt. Osmoregulation in birds is accomplished in part by renal mechanisms, including their ability to modulate renal filtration (glomerular filtration rate, GFR) and tubular water reabsorption. The excretion of nitrogenous wastes primarily as uric acid (or urate salts) also provides a means of conserving water (Braun, 2003). Beyond this, osmoregulation in birds depends on other organs that regulate salt and water losses. When the urinary and digestive systems share a common opening, the cloaca (found in birds and some amphibians and reptiles), conservation of water and ions can be achieved by refluxing urine from the cloaca along the hindgut. As discussed above, this retrograde movement of urine into the colon (rectum) and digestive caecae (when present) may also be important for nitrogen and energy balance, the former especially in birds with low-N diets such as hummingbirds, honeyeaters, rock ptarmigan *Lagopus mutus* and grouse of artic regions (Roxburgh & Pinshow, 2002; Skadhauge, 1981). In this section we focus on the role of the lower intestine in water and salt regulation [also recently reviewed by Braun (2003) and Goldstein & Skadhauge (2000)].

The hindgut (coprodeum, colon, caeca) of birds is capable of active sodium reabsorption and solute-linked water absorption (Braun, 2003; Laverty & Skadhauge, 1999; Singer, 2003; Thomas, 1982) and can thus modify both the composition and volume of refluxed urine. At least some water can be removed from hyperosmotic urine in birds (Skadhauge, 1980). The details of this absorption have mostly been studied in the coprodeum (Skadhauge, 1981), rectum (Goldstein, 1989b), and caecum (Goldstein, 1989*a*; Thomas & Skadhauge, 1989*b*) of birds (mostly domestic fowl, presumably the mechanisms are similar in wild species). In all avian species examined to date, the epithelium of the lower intestine reabsorbs Na^+, Cl^-, and water from isotonic saline perfusion solutions by mechanisms similar to those identified in mammals (Goldstein, 1989*b*; Goldstein & Skadhauge, 2000). Note that tightly regulated control of salt and water balance in the avian lower intestine (Goldstein & Skadhauge, 2000; Laverty *et al.*, 2006) does not conflict with the finding of significant paracellular nutrient absorption (implying relatively higher epithelial permeability) in the small intestine (see Section V). Epithelial permeability decreases along the length of the intestine, the distal colon of mammals (Powell, 1987) and coprodeum of birds (Goldstein & Skadhauge, 2000) being the least permeable regions.

In the domestic fowl the nominal (serosal) surface area of the caeca is of equal magnitude to that of the coprodeum and colon, but Na^+ and water transport rates per unit body mass are approximately threefold higher (Thomas & Skadhauge, 1989*a*). Caecal transport capacity for NaCl and water from chymus and refluxed urine is therefore probably large, perhaps larger than that of the colon plus coprodeum. But the relative importance of the caecum is not always apparent, because ligation of the caeca in

hydrated birds appears to have little overall effect on osmoregulation (Anderson & Braun, 1984; Braun & Duke, 1989; Skadhauge, 1981; Williams & Braun, 1996). Possibly, this is because of compensation by other organs (Hughes, Kojwang & Zenteno-Savin, 1992; Williams & Braun, 1996), and the osmoregulatory contribution of the caeca may therefore become essential only during the combined stresses of food, water and salt depletion (Thomas, 1982; Thomas & Skadhauge, 1989*a*).

The transport of salt and water across the avian hindgut appears to be hormonally regulated; both mineralocorticoids and glucocorticoids have been implicated (Laverty & Skadhauge, 1999). In particular, electrogenic Na^+ channel activity appears to be modulated by plasma levels of aldosterone. Experiments in which saline solutions were infused into either the cloaca or the carotid artery of domestic fowl suggested that the composition of urine is sensed within the cloaca, implying local control of refluxing action (Brummermann & Braun, 1995). This system would prevent extracellular fluid loss to the colon should the contents become too concentrated (Braun, 2003).

(3) Variation in the integrated functioning of the kidney and gut

Avian hindgut regions play variously important roles in water and salt balance depending on ecological factors (environment, diet) and physiological factors (Na^+ and water balance). Although the mechanisms for salt and water transport may be broadly similar within the GIT and among species, what differs dramatically among species is the extent to which these regions are important at the whole-animal level. The latter is a function of their surface areas and the flow rates and composition of fluid through them (Goldstein & Skadhauge, 2000; Karasov & Hume, 1997). This is illustrated in several comparisons of how relatively closely related avian species can handle the challenges of water and sodium under- and over-abundance very differently.

(*a*) *Response to dehydration*

Consider, for example, four species that inhabit arid or semi-arid environments: ostrich, sand partridges *Ammoperdix heyi*, Gambel's quail, and emu (Goldstein, 1989*b*). The former two species produce hyperosmotic urine and exhibit little retrograde movement of urine when dehydrated. By contrast, the emu (a ratite, like the ostrich) produces weakly concentrated urine but compensates with a greatly enhanced capacity for postrenal reabsorption in the lower intestine; the surface area of its rectum is large and the tissue exhibits a high tissue-specific Na^+ uptake rate. Gambel's quail differs from the sand partridge (both are phasianids) in exhibiting considerable postrenal modification of ureteral urine in the coprodeum, rectum, and possibly caecum. In these examples, the role of the hindgut in osmoregulation is not according to taxonomic affiliation but according to the roles of other components of the integrated renal-GI system (Karasov & Hume, 1997).

(*b*) *Response to high salt loads*

Another example concerns the response of duck species to excess Na^+ levels, which may be ingested when feeding and drinking in marine environments. Schmidt-Nielsen *et al.* (1963) postulated that the hindgut would be particularly important for osmoregulation in birds with salt glands exposed to high salt loads: ureteral urine might be refluxed, NaCl reabsorbed and recycled to salt glands, and solute-linked water conserved. In the glaucous-winged gull *Larus glaucescens*, a true marine bird, reflux and modification of already hyperosmotic ureteral urine seems relatively unimportant in overall osmoregulation (Goldstein, 1989*b*). In NaCl-loaded ducks, which also possess salt glands and excrete hyperosmotic ureteral urine, the extent to which hindgut recycling of ureteral urine occurs is also unclear (Bennett & Hughes, 2003; Hughes & Roberts, 1988). The importance of the hindgut for Na^+ regulation among duck species probably depends on diet and habitat associations. Bennett & Hughes (2003) recently found that renal function (GFR and renal fractional water and Na^+ recovery) in three species of ducks was little affected by saline acclimation or by acute saline loading. They found that the Barrow's goldeneye (*Bucephala islandica*), a marine-adapted species, was able to completely excrete an infused Na^+ load *via* salt glands, while the mallard (*Anas platyrhynchos*), usually a freshwater species, used a combination of salt glands and decreased renal (or postrenal) Na^+ recovery associated with additional urinary water loss to eliminate an infused Na^+ load. By contrast, the canvasback (*Aythya valisineria*) was unable to completely eliminate an infused Na^+ load, yet tolerated higher drinking water salinities than the mallard (Bennett, 2002). This suggests that in canvasbacks water and Na^+ regulation are carried out by organs other than the kidneys and salt glands, and thus that the hindgut plays a relatively more important osmoregulatory role.

For birds lacking salt glands but still exposed to high salt loads, Thomas (1982) argued that the lower intestine would not be of much use for water conservation because water cannot be reclaimed without absorbing NaCl. Indeed, Lotz & Martínez del Rio (2004) recently found that nectarivorous rufous hummingbirds (*Selasphorus rufus*) are able to tolerate only very low dietary electrolyte concentrations. Using the medical terminology, these hummingbirds appear to be salt-sensitive (Espinel, 1992). They retained steadily increasing amounts of Na^+ and Cl^- as their dietary NaCl concentration increased above 35 mmol l^{-1}. Lotz and Martínez del Rio (2004) concluded that this salt sensitivity must be caused by the poor ability of their kidneys and lower (distal) GIT to concentrate urine, probably a result of evolutionary (ultimate) adaptation to a watery and electrolyte-poor diet.

(*c*) *Response to low salt load*

On a low-NaCl diet the domestic fowl coprodeum absorbs Na^+ at a very high rate, about 100 μeq (kg body mass)$^{-1}$ h^{-1} *in vivo* (see Goldstein & Skadhauge, 2000, and references therein). Na^+ transport by the coprodeum may

be completely supressed by NaCl loading, as no transport of nutrients (glucose, amino acids) has been measured there and neither can a solute-linked water flow be detected *in vivo* (Rice & Skadhauge, 1982b) or *in vitro* (Bindslev, 1981). Colon from birds (domestic fowl and galah *Cacatua roseicapilla*) maintained on a low-NaCl diet has a Na^+-absorption capacity similar to coprodeum, particularly an absence of stimulation of Na^+ transport induced by hexoses or amino acids (Goldstein & Skadhauge, 2000). For a high NaCl intake colonic transport differs remarkably from that of coprodeum: rather than being suppressed, Na^+ absorption continues at about half the rate observed in coprodeum during NaCl limitation, but only when glucose and amino acids are present in the lumen (Clauss, Dantzler & Skadhauge, 1991; Lind *et al.*, 1980*b*; Rice & Skadhauge, 1982*a*). This transport is not affected by the blocker of apical Na^+ channels amiloride, which totally suppresses Na^+ absorption in both coprodeum and colon from NaCl-depleted birds. Salt loading appears to induce a switchover in the avian colon from a Na^+ channel to Na^+/nutrient cotransport (Goldstein & Skadhauge, 2000). Although there are fewer studies on the avian caeca, the available evidence suggests broad qualitative similarities among the species examined (Goldstein, 1989*a*). In the domestic fowl, there are many qualitative similarities between rectocoprodeal and caecal function: (1) primary importance of active Na^+ absorption, (2) occurrence of Na^+-linked components of water and Cl^- absorption and K^+ secretion, and (3) similar responses to levels of dietary Na^+, glucose and exogenous aldosterone.

The effects of NaCl deprivation on hindgut salt and water fluxes have been studied most intensively in the domestic fowl. NaCl depletion causes increases in amiloride-sensitive Na^+ transport in the rectocoprodeum, with a half-time of acclimation of 2–4 days (Thomas & Skadhauge, 1982). In the domestic duck, NaCl depletion also causes increases in amiloride-sensitive Na^+ transport, though relative to the chicken rates are lower. The acclimational changes in transport capacity may be associated with movement and activation of nascent Na^+ channels in the cytoplasm and the apical membrane (Goldstein, 1989*b*). Thus, Na^+ deprivation results in potentially homeostatic increases in the Na^+-transporting capacity of the whole hindgut. While the deprivation is associated with elevated endogenous aldosterone levels, exogenous aldosterone reproduces only some of the effects of low-Na^+ diets on rectocoprodeal or caecal function (Thomas & Skadhauge, 1988).

(4) An example of integrated responses

We began this section on the role of the hindgut in osmoregulation with a discourse on nectarivores, and we will also close with them because they exemplify integrated responses so well. The unique diet of these animals requires an integrated response to simultaneous challenges in both the water and Na^+ budgets, and the responses are an integration of processes in the gut and kidney.

Nectar-feeding birds are unusual among terrestrial animals in that they often ingest and excrete prodigious water volumes to obtain adequate energy. Their nectar diets are also electrolyte poor (Calder & Hiebert, 1983), thus they confront the unusual challenge of having to conserve electrolytes. Lotz & Martínez del Rio (2004) studied the ability of rufous hummingbirds to conserve electrolytes when fed electrolyte-poor diets. They found that the kidneys of these hummingbirds had a remarkable ability to produce dilute urine, but that they were able to excrete fluid even more Na^+-dilute than their ureteral urine. When fed electrolyte-free diets, rufous hummingbirds could produce excreta containing only 0.4 and 0.2 mmol l^{-1} respectively of Na^+ and K^+. This was presumably because of Na^+ reabsorption in the hindgut. By comparing urinary and excreted fluid Na^+ outputs, these authors estimated that a significant fraction of daily renal Na^+ loss (38%) was apparently recovered by the hindgut. In spite of excreting large volumes of fluid (twice their body mass daily) hummingbirds fed on electrolyte-free diets lost electrolytes at very low rates. Similarly, Goldstein & Bradshaw (1998) found urinary Na^+ excretion rates in excess of intake rates in nectarivorous red wattlebirds (*Anthochaera carunculata*), suggesting substantial post-renal Na^+ reabsorption in the lower intestine. Palestine sunbirds appear to rely on the integrated functioning of two organ systems to maintain water balance in spite of highly variable and often extremely high water intake rates: (1) fractional absorption of dietary water is modulated in the GIT (McWhorter *et al.*, 2003*a*) and (2) renal filtration rate (GFR) is low and relatively insensitive to water loading, but water recovery is modulated by the kidney (McWhorter *et al.*, 2004). McWhorter *et al.* (2004) found that Palestine sunbirds excreted fluid that had lower total osmotic and glucose concentrations than their ureteral urine. Although these authors concluded that this was probably the result of dilution of urine with unabsorbed dietary water that is shunted through the gut in this species (McWhorter *et al.*, 2003*a*), it is also probable that some electrolyte and glucose recovery occurred in the lower GIT. The remarkable combined renal and gastrointestinal electrolyte-conserving abilities of nectar-feeding birds play an important role in allowing them to cope with watery, electrolyte-poor diets and are likely the result of evolutionary adaptation to these diets.

VII. CONCLUSIONS

(1) Mathematical chemical reactor modeling of digestive capacity, integrating the maximal reaction velocity (V_{max}) of intestinal hydrolases or nutrient transporters along the length of the intestine to yield total hydrolytic or transport capacity, has been and will continue to be a useful approach. The data available so far seem to indicate that birds have lower capacities for hydrolysis and mediated absorption than do similar sized mammals because they have smaller intestines. Yet, birds can exhibit digestive efficiencies comparable to those of mammals despite taking in relatively more food per day and processing in relatively shorter time. One hypothesis

is that birds have less spare digestive capacity than do mammals.

(2) The caecum is the important site of fermentation in most herbivorous avian species, resulting in the availability of both energy, in the form of short chain fatty acids (SCFAs), and essential nutrients (vitamins, amino acids). However, direct quantitative data on coprophagy in wild avian herbivores and omnivores, as exists for mammals, is lacking. Capacities for caecal/hindgut amino acid and vitamin transport in relation to the capacity in the small intestine, minimum requirements, or daily inputs are not well understood. Similarly, it is not clear whether N recycling that truly improves host N economy occurs, or whether endogenous or microbial breakdown of protein to release essential amino acids is more important in the hindgut.

(3) Paracellular (non-mediated) nutrient absorption provides a parallel pathway to mediated absorption of sugars and amino acids and thus increases the small intestine's absorptive capacity. The enhancement of paracellular absorption in birds relative to mammals appears to partially compensate for typically shorter intestines in birds, but may also increase vulnerability to water soluble toxins and thus influence feeding behaviour and dietary breadth.

(4) The avian intestine plays an important role in salt and water regulation. Apparent adaptive regulation of absorption of ingested water across the GIT has been tested in three species of nectar-feeding birds (two hummingbirds and a sunbird) and found in one (the sunbird), so more studies are in order. Because of the mixing of urinary and digestive material in the cloaca, the avian hindgut plays a more significant role in salt and water regulation than in mammals. The importance of this role varies depending on ecological factors (environment, diet) and physiological factors (salt and water balance).

(5) A rarely explored question is whether and how regulation of structure and physiology of the gut is a compromise between digestion and protection. Do the well documented changes in the GIT resulting from various nutritional states such as hyperphagia or food restriction (see Introduction) affect gut immune function (Fassbinder-Orth & Karasov 2006)? Can digestive and immune responses of the gut be regulated independently? Could birds, with their well documented phenotypically plastic guts be good models for studying these questions? We think so. Baker *et al.* (2004) provide an excellent example of a system (the migratory red knot, *Calidris canutus*) in which simultaneous tests of immune and digestive parameters would shed light on concomitant or opposite changes in function.

VIII. ACKNOWLEDGMENTS

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