Digestive Challenges for Vertebrate Animals: Microbial Diversity, Cardiorespiratory Coupling, and Dietary Specialization*

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ABSTRACT

The digestive system is the interface between the supply of food for an animal and the demand for energy and nutrients to maintain the body, to grow, and to reproduce. Digestive systems are not morphologically static but rather dynamically respond to changes in the physical and chemical characteristics of the diet and the level of food intake. In this article, we discuss three themes that affect the ability of an animal to alter digestive function in relation to novel substrates and changing food supply: (1) the fermentative digestion in herbivores, (2) the integration of cardiopulmonary and digestive functions, and (3) the evolution of dietary specialization. Herbivores consume, digest, and detoxify complex diets by using a wide variety of enzymes expressed by bacteria, predominantly in the phyla Firmicutes and Bacteroidetes. Carnivores, such as snakes that feed intermittently, sometimes process very large meals that require compensatory adjustments in blood flow, acid secretion, and regulation of acid-base homeostasis. Snakes and birds that specialize in simple diets of prey or nectar retain their ability to digest a wider selection of prey. The digestive system continues to be of interest to comparative physiologists because of its plasticity, both phenotypic and evolutionary, and because of its widespread integration with other physiological systems, including thermoregulation, circulation, ventilation, homeostasis, immunity, and reproduction.

Introduction

An army marches on its stomach. (A proverb or quote often attributed to Napoleon Bonaparte or Frederick the Great)

The annual migration of wildebeest and zebra around the Serengeti ecosystem is just one of many "armies" of fish, birds, reptiles, and mammals that follow the availability of food as plant production changes with seasonal patterns of precipitation and temperature. Plants support prey, which in turn support predators: grasses feed wildebeest, zebra, and gazelle that are consumed by lions and crocodiles; seeds and flowers feed rodents and birds that are consumed by raptors and monitor lizards. The digestive system is the interface between the supply of food for an animal and the demand of energy and nutrients for completing its life history. Differences in the digestive system have long been proposed as a mechanism for partitioning niche among African herbivores (Bell 1971; Demment and Van Soest 1985; Hofmann 1989). Food intake varies with both the abundance of food and the demands of the animal for maintaining its body and growing new tissue. Consequently, the digestive system must accommodate and degrade a wide range of substrate loads as animals increase food intake to complete each stage of growth and reproduction, during seasons of plant growth, or to survive the ensuing periods of low food abundance when plants are dormant or senescent. The close relationship between life history and nutrition is often reflected in the timing of breeding: ungulates in the Serengeti synchronize birth with either peak plant growth or peak plant biomass (Sinclair et al. 2000); European songbirds that migrate over

short distances begin nesting earlier when warm weather advances plant growth and allows insect prey to emerge earlier in spring (Both et al. 2004).

Food selection often changes with seasonal or regional variation in food abundance. These dietary changes may therefore alter both the amount and the type of substrates that are digested, absorbed, and processed. Plant diets are usually more difficult to process than animal diets. Carnivores consume tissues that can be quickly degraded by endogenous enzymes, whereas herbivores consume a wide array of complex carbohydrates and cell walls that are slow to degrade and often require exogenous enzymes from microbes. Herbivores may also ingest toxins that impair digestion and increase the costs of processing nutrients. The digestive tracts of carnivores are structured for the physical and chemical disruption of food by grinding and acidification in the foregut (e.g., in the antral mill of dogs; Malagelada and Azpiroz 1989). Conversely, the digestive tracts of herbivores emphasize the accommodation of plant fiber for microbial fermentation in the foregut and the hindgut (Barboza et al. 2009). Digestive and metabolic costs reduce the amount of energy, nutrients, and water gained from the diet, that is, the net return or metabolizable intake of each component after accounting for losses to excretion and respiration (Hume 2005; Barboza et al. 2009). Net returns may fall below requirements when animals ingest small meals of low-quality foods or incur excessive loads of metabolites and toxins. Consequently, some animals may reduce the costs of maintaining a digestive system when feeding is interrupted during periods of low food abundance such as the winter hibernation of rodents (Carey 2005).

Digestive systems are not static morphological attributes but dynamic systems that respond to changes in the physical and chemical characteristics of the diet and the level of food intake (Starck 2005). Consequently, animals may alter the efficiency and capacity of their digestive systems to respond to changes in available food and their requirements for energy and nutrients. The normal range of digestive response is enhanced by "spare capacity" in volume and rates of secretion and transport; spare capacities increase digestible intakes by 9%–50% when requirements are suddenly increased for thermoregulation in small birds and mammals (Karasov and McWilliams 2005). However, more time may be required to adjust complex digestive processes such as fermentation or detoxification when diets are altered for herbivores. Here we discuss physiological adaptations that affect the ability of an animal to optimize digestive function in relation to novel substrates and changing food supply: fermentative digestion in herbivores, integration of cardiopulmonary and digestive functions, and dietary specialization. These digestive adaptations were discussed in a session of seven presentations entitled "Integrative Comparative Physiology of Digestion" at the Fourth International Conference on Comparative Physiology and Biochemistry (ICCPB4; Maasai Mara National Reserve, Kenya). That session and this review progresses from digestion with exogenous enzymes of microbial symbionts to digestion with endogenous enzymes and processes of the animal.

Fermentative Digestion and Microbial Diversity

Microbes colonize the mucosal surface of the digestive tract as well as the fluids and particles of digesta to form a closely integrated ecological unit with the host. This diverse mixed culture mainly comprises bacteria but also includes methanogenic Archaea, ciliate and flagellate protozoa, anaerobic phycomycete fungi, and bacteriophages. The gut microbiome can be considered the most metabolically active and rapidly renewable organ of the body (Mackie and Cann 2005). Studies of the gut microbiome face two major limitations: (1) the inevitable bias introduced by laboratory culture-based enumeration and characterization and (2) the lack of a phylogenetically based classification scheme. These limitations can be overcome by using techniques based on the analysis of nucleic acid sequences for common functional units such as the ribosome and by quantification of these sequence-based targets (Woese 2002; Smalla 2004). Recent studies of nucleic acid sequences have demonstrated that the gut microbiome is much more diverse than previously predicted by laboratory culture-based techniques. For example, only 11% of bacterial taxa detected by nucleic acid sequences have been cultured from the foregut of domestic ruminants even though the ruminal microbiome has been intensively studied over the past century (Edwards et al. 2008). Taxonomic comparisons of microbes are more conservative than those of their vertebrate hosts. Bacteria are grouped within the same phylotype or operational taxonomic unit (OTU) when the match between a targeted sequence (e.g., the gene for the 16S subunit of the ribosome [the 16S rRNA gene]) is greater than 97%, whereas vertebrate species share much more than 99% of the same sequence (Staley 2004). The functional diversity of the gut microbiome far exceeds that of the host because the microbes outnumber both the cells and the genes of the animal host by a factor of 100 (Attwood et al. 2008).

All vertebrates are hatched or born germ free, but the gut is soon colonized by microbes as the neonate comes into contact with food and the saliva, feces, and skin of other animals (Conway 1997; Mead 1997). However, microbial colonization of the gut is not random; microbes that become established in the vertebrate gut are taxonomically distinct from those on the surface of the animal and in its environment (Ley et al. 2008b). Furthermore, only two bacterial phyla (Firmicutes and Bacteriodetes) predominate over the 50 bacterial phyla that could possibly inhabit the gut of mammals (Eckburg et al. 2005; Dethlefsen et al. 2006; Ley et al. 2008a). Microbial colonization of the gut was recently examined by transferring inocula from zebrafish (*Danio rerio*) into germ-free mice (*Mus musculus*) and from mice into germ-free zebrafish (Rawls et al. 2006). Colonization is not passive but is shaped by the host: Firmicutes that predominated in donor mice were able to colonize the germ-free zebrafish, but Fusobacteria that were typical of the zebrafish community became more abundant in the germ-free fish than in their donor mice. The gut microbiome therefore tends toward a community structure typical of the host species, which is consistent with parallel selection or coevolution among

microbes and their host. At ICCPB4, Mackie et al. (2008) presented a comparison of the microbiomes of marine iguanas (*Amblyrynchus cristatus*) and land iguanas (*Conolophus subcristatus* and *Conolophus pallidus*) on the Galápagos Islands. Their study demonstrated that all three species host a novel group of archaebacteria that is not present in other herbivorous reptiles, including tortoises (*Testudo elephantophus*) from the same islands or green iguanas (*Iguana iguana*) from Panama. These observations suggest that the novel methanogens may have coevolved with iguanas on these remote islands because land and marine iguanas now use very different habitats and diets on the Galápagos (Mackie et al. 2008).

The relationship between microbes and the host depends on the habitat used by the microbe in the digestive tract. Microbes that are primarily associated with the mucosal lining of the tract have the closest relationship with their host species. Secretions of antibodies can suppress potential pathogens while secretions of mucins and lectins foster more benign microbes that can competitively exclude potential pathogens from the mucosa (Klasing 2005; Cash et al. 2006). Variation in the gut microbiome between individual hosts is 10%–25% even when the hosts are genetically similar (e.g., monozygotic twins), live under the same conditions, and consume the same diet, because the history of colonizing the gut and the secretions of the host also vary between individuals (Dethlefsen et al. 2006).

The diversity of the mammalian gut microbiome is greater in herbivores than in carnivores (Ley et al. 2008*a*) because the number of substrates and habitats within the digestive tract also expand as the host uses the structural components of plants. Gut microbes depend on the host for conditions such as neutral pH and low oxygen tension as well as the nutrients and the retention time required to complete their life cycles. Consequently, numbers of microbes are low in gastric regions where digesta pH is low and digesta flow is high. Bears, dogs, and pandas have simple digestive tracts with short retention times that provide little time for establishment of slow-growing microbes and the fermentation of structural carbohydrates. Therefore, the microbiome of the herbivorous giant panda (*Ailuropoda melanoleuca*) is more similar to the simple communities found in other mammals in the order Carnivora than the complex assemblages found in the foregut and hindgut fermentations of ruminants (order Artiodactyla) and horses (order Perissodactyla; Wei et al. 2007; Ley et al. 2008*b*).

The number and composition of microbes in foregut and hindgut fermentation depend on complementary changes in diet and digestive function. Rapid changes in carbohydrate consumption can alter both the microbial community and the acids they produce, resulting in distension of the foregut (bloat) in ruminants and both diarrhea and damage to connective tissues (laminitis) in ruminants and horses (Russell and Rychlik 2001; Milinovich et al. 2008). However, ungulates do alter their food intake gradually through the year, especially where patterns of plant growth vary with season. Microbial diversity therefore changes with season as both the amount and the composition of substrates are altered. The number of cellulolytic bacteria in the rumen of reindeer (*Rangifer tarandus platyrhynchus*) follows the seasonal pattern of plant fiber content, which is high in winter and low in summer (Orpin et al. 1985). High intakes and a wide selection of forages during summer result in a diverse ruminal microbiome in reindeer (Sundset et al. 2009), with a bacterial population richness estimated at as much as 698 OTUs, which is larger than the number of bacterial phylotypes (300–400 OTUs) reported for domestic ruminants (Sundset et al. 2007, 2009; Edwards et al. 2008).

Digestion of structural polysaccharides from plants is the basis of the mutualism between microbes and vertebrate herbivores because unlike some invertebrates, vertebrate animals do not express hydrolases for cellulose and hemicellulose (Linton and Greenaway 2007). However, cellulolytic function of the rumen is due to only a few species in the genera *Fibrobacter* and *Ruminococcus* that use different mechanisms for attachment to the cell wall (Flint and Bayer 2008; Wallace 2008). Cellulose degradation is slow and facilitated by long retention times in large volumes of digesta. At ICCPB4, Barboza and Forster presented a seasonal comparison of the ruminal fermentation in muskoxen (*Ovibos moschatus*) that combined changes in both digestive function and microbial activity for fibrous forages at high intakes. They observed that cellulose degradation rates double between spring and autumn as animals increase their ruminal volume but conserve the time for retention of fiber particles in the rumen (Peltier et al. 2003; Barboza et al. 2006). Conversely, microbial numbers and activity decline in winter when food intakes of muskoxen are low. Diurnal and postprandial variation in rumen pH also increases in winter for muskoxen, indicating that less energy is expended in regulating ruminal conditions for a smaller community of microbes with low returns of energy and protein for the host (Crater and Barboza 2007; Crater et al. 2007). Recent estimates of diversity indicate a fourfold increase in bacterial phylotypes within the rumen as the muskox increases food intake and ruminal "services" for the microbiome between spring and autumn (R. J. Forster and P. S. Barboza, unpublished data).

A large and diverse microbiome such as the rumen may retain dormant phylotypes that are able to exploit changes in conditions and substrates by expressing novel enzymes. Bacteria that can quickly use new substrates to replicate rapidly have a high capacity for protein synthesis (Dethlefsen and Schmidt 2007). In contrast, substrates that provide a low rate of energy return may support only slow-growing phyla that depend on other microbes to provide complementary pathways and substrates for their replication (Russell and Rychlik 2001). Microbes that can tolerate and detoxify plant secondary metabolites may be mutually beneficial to the microbiome and the host and are thus more likely to arise in diverse communities with functional redundancy, that is, communities in which more than one organism can provide the same service (Ley et al. 2006). Organisms that express novel services such as detoxification may be rare but readily established due to the high selective pressure on the host and the microbiome. For example, the bacterium *Synergestes jonesii* detoxifies the Indonesian forage *Leucaena leucocephala* in resident goats (*Capra hircus*). Transfer of the bacterium to Australian goats and cattle (*Bos*

taurus) conferred the same detoxification to the new hosts (Allison et al. 1992; Wallace 2008). Similarly, prolonged exposure of Norwegian reindeer to phenolic secondary components in lichens has favored a bacterium (*Eubacterium rangiferina*) that is resistant to the antibiotic usnic acid (Sundset et al. 2008), one of several toxins in lichen that can be fatal to sheep (*Ovis aries*) and elk (*Cervus canadensis*; Dailey et al. 2008). At ICCPB4, Sundset and coworkers presented data from digestibility studies that also confirm the microbial degradation of usnic acid in the rumen of reindeer fed lichens (Sundset et al. 2010). Many plants eaten by ruminants on their natural pastures contain plant polyphenolic compounds such as tannins. Dietary tannins depress food intake, fiber digestibility, and postruminal degradation of protein in ruminants (McAllister et al. 1994; Barry and McNabb 1999; Hervás et al. 2000, 2003; Silanikove et al. 2001). Tannins form complexes with proteins, which may have an advantageous effect in domestic ruminants feeding on diets that are high in proteins and energy by decreasing the availability of soluble protein to microbial degradation in the rumen and hence allowing the protein to bypass the rumen (Tanner et al. 1994). Although tannins have antimicrobial effects, tannin tolerance and degradation have been reported in bacteria from the gastrointestinal system of various ruminants, koalas (*Phascolarctos cinereus*), and humans (Nelson et al. 1998; Odenyo and Osuji 1998; Goel et al. 2005). Consumption of tannins has been shown to increase the counts of tannin-resistant bacteria in the gut by selecting for resistant species of *Enterobacteriaceae* and *Bacteroides* (Gram-negative groups of bacteria) in rats (Smith and Mackie 2004). Tannin-resistant rumen bacteria may protect ruminants from the antinutritional effects of a diet rich in tannins. The inhibitory effect of tannins on gastrointestinal bacteria seems to be related to the ability of these tannins to form complexes with polymers and minerals. Bacteria may overcome this inhibition by degrading the tannin itself, dissociating the tannin from the substrate complexes, inactivating the tannin by high-affinity binders, modifying/repairing the membrane, or sequestering metal ions (Smith et al. 2005). Tannin resistance strategies in *Escherichia coli* were recently reported to include the upregulation of cell envelope stress protein genes and multidrug transporter-encoding operons in the presence of tannins (Zoetendal et al. 2008). Herbivores that select a variety of forages with different toxins and nutrients may be able to maintain detoxification systems in microbes and their tissues that allow the animal to tolerate toxins especially when food abundance declines and food choices are limited to those plants that are heavily defended with antinutrients (Foley et al. 1999; Makkar 2003).

Integration of Cardiorespiratory and Gastrointestinal Performance in a Model System

Ingestion of a meal sparks an array of integrated responses that serve to break down, absorb, and assimilate the nutrients of the meal and remove undigested residues. Neural, humoral, and luminal signals from the tissues of the gastrointestinal tract set into play cascades of energy-consuming cellular processes to digest the meal. The resulting postprandial increase in gut tissue metabolism is accompanied at the whole-animal level by appropriate increments in ventilatory and cardiovascular responses. In general, physiological responses to digestion and the cellular mechanisms of meal breakdown and absorption are similar among vertebrates as well as most invertebrates (Karasov and Martínez del Rio 2007). For both invertebrates and vertebrates, feeding commonly generates the specific dynamic action (SDA) of the meal (Secor 2009), a 50%–300% increase in metabolic rate. Mechanisms of intestinal epithelial transport are likewise shared among a large diversity of invertebrate and vertebrate species (Karasov and Hume 1997; Wright and Ahearn 1997). Studies of digestive physiology have found that vertebrates in general regulate gastrointestinal and cardiorespiratory performance to a modest extent between fasting and feeding. One exception is the unprecedented magnitude of postprandial responses exhibited by intermittently feeding snakes (e.g., species of boas and pythons). Because these responses are so large and prolonged, they are excellent experimental systems in which to study how cardiorespiratory and gastrointestinal responses are integrated.

Swallowing and the arrival of the meal into the stomach trigger the production of HCl and the protease pepsin. Whereas most other vertebrates apparently maintain a baseline production of HCl between meals and, hence, an acidic gastric environment at all times, the python ceases HCl production during fasting but rapidly upregulates HCl secretion upon ingestion (Secor 2003). The python's large and intact meals require an elevated and continuous level of HCl production lasting as long as a week or more. Gastric metabolism is elevated because of the increased activity of H^+/K^+-ATPase (i.e., proton pumps) that transports H^+ out of the oxyntopeptic cells (parietal cells for mammals) against a tremendous concentration gradient at the expense of one ATP per H^+ pumped (Reenstra and Forte 1981; Helander and Keeling 1993). Evidence that gastric performance has a strong metabolic signal includes the findings that smaller and more easily digested meals generate a lower metabolic response and that the experimental depression of HCl production after feeding (with the proton-pump inhibitor omeprazole) temporarily suppresses the postprandial metabolic response of the *Boa constrictor* (Secor and Diamond 1997; Secor 2003; Andrade et al. 2004).

For pythons, the extended duration of their gastric acid production has a profound impact on their acid-base balance. With HCl production, plasma Cl^- is exchanged for HCO_3^-, thereby increasing plasma $[HCO_3^-]$, hence alkalizing the plasma and generating an "alkaline tide" (Overgaard et al. 1999; Wang et al. 2001*a*; Skovgaard and Wang 2008). Interestingly, arterial pH of pythons and other air-breathing vertebrates remains relatively stable after feeding because the rise in plasma $[HCO_3^-]$ is attended by an elevation of arterial P_{CO_2} (Overgaard et al. 1999; Busk et al. 2000*a*, 2000*b*; Wang et al. 2001*a*). The postprandial elevation of arterial P_{CO_2} is caused by hypoventilation, whereby effective lung ventilation does not increase proportionally with CO_2 production (Wang et al. 2001*a*). This ven-

tilatory compensation of the alkaline tide presumably protects tissues from acid-base imbalance (Skovgaard and Wang 2008).

When sufficient gastric breakdown has occurred, the acidic chyme is metered through the pyloric sphincter into the small intestine and is immediately met by bile and pancreatic secretions. Pancreatic HCO_3^- neutralizes the acidic chyme as demonstrated by the immediate restoration of luminal pH (from 2 to 6 units) within the first centimeters of the small intestine (Secor et al. 2006). Bile, pancreatic lipases, amylase, and proteases continue the breakdown of macromolecules within the small intestine. For the python, pancreatic performance is enhanced during feeding by increasing pancreatic mass (by two times) and enzyme activity (by five to 20 times; Cox and Secor 2008).

It is characteristic of the vertebrate intestine to experience a 25%–50% increase in epithelial mass and potential modest increases (more than two times) in function following feeding after a period of fasting (Dunel-Erb et al. 2001; Secor 2005a). In contrast, after feeding, intermittently feeding snakes and estivating anurans experience a two- to threefold increase in small intestinal mass and a two- to 10-fold increase in mass-specific rates of intestinal nutrient transport and hydrolase activities. These responses are rapidly reversed following the completion of digestion (Secor 2005a, 2005b; Ott and Secor 2007). Although these regulatory responses are impressive, they probably contribute little to the animal's large postprandial increase in metabolic rate (Overgaard et al. 2002; Secor 2003). Counter to the intestinal epithelium, the muscular layer of the intestine, as well as intestinal motility and its responses to regulatory peptides, remain unaffected by fasting and digestion (Holmberg et al. 2002). For pythons, a cellular mechanism underlying the wide regulation of their intestinal function is the rapid postprandial lengthening (by five times within 24 h) of their intestinal microvilli after feeding, and their subsequent shortening following digestion (Lignot et al. 2005; Ott and Secor 2007). Assuming that the density and activities of membrane transporters and hydrolases do not change with feeding, the increase and subsequent decrease in microvillus length and, hence, apical surface area could largely explain the up- and downregulation of intestinal function. Supporting the link between intestinal form and function are the findings presented by Secor and Lignot at ICCPB4: amphibians and reptiles that do not modulate microvillus length with feeding and fasting do not experience significant regulation of intestinal nutrient transport (S. M. Secor and J.-H. Lignot, unpublished data). Digestion is supported by an increase in cardiac output that elevates the perfusion of the active gut. The rise in gastrointestinal blood flow (i.e., postprandial hyperemia) supplies additional oxygen and nutrients to tissues, serves to remove metabolic waste (e.g., CO_2 and NH_3), provides necessary building blocks for tissue growth (e.g., amino acids), transports absorbed nutrients from the intestine to the liver, and delivers regulatory peptides (i.e., hormones) to appropriate tissues (Fara 1984; Farrell et al. 2001; Pappenheimer and Michel 2003). For the majority of the vertebrates studied, feeding stimulates a 25%–50% increase in cardiac output and a 50%–150% increase in gastrointestinal blood

flow (Axelsson and Fritsche 1991; Sidery et al. 1991; Altimiras et al. 2008). In concert with their large metabolic response and digestive demand, Burmese pythons (*Python molurus*) experience a fivefold increase in cardiac output and an 11-fold increase in superior mesenteric blood flow during the digestion of meals weighing 25% of their body mass (Secor and White 2007). If forced to crawl while digesting, cardiac output is further elevated but superior mesenteric flow is severely depressed (Secor et al. 2000a; Secor and White 2007). The postprandial increase in cardiac output stems primarily from a 3.5-fold increase in heart rate (postprandial tachycardia) and, secondarily, from a 45% increase in stroke volume, the latter being contributed to by a 40% increase in ventricular mass (Andersen et al. 2005; Secor and White 2007). The postprandial tachycardia is caused by withdrawal of vagal tone to the heart but also from what appears to be a direct stimulation of circulating hormones or signal molecules (Wang et al. 2001b; Skovgaard and Wang 2008).

The kaleidoscope of metabolic, morphologic, and functional responses from onset to completion of digestion is triggered by an intricate array of neural, humoral, and luminal signals. For the Burmese python and probably for other intermittently feeding snakes, feeding results in rapid postprandial increase (by as much as 25 times) in plasma concentrations of cholecystokinin, glucagon, glucose-dependent insulinotrophic peptide, insulin, and neurotensin (Secor et al. 2001; Secor 2008). The specific role that each of these peptides plays in python digestion remains largely unknown, but several neuropeptides (substance P, neuropeptide γ, and neurotensin) have been demonstrated to increase cardiac output or dilate mesenteric vessels for pythons (Wang et al. 2000; Skovgaard et al. 2005, 2007). Initiated by feeding and the gross distension of the gastric wall, neurally mediated signals are expected to underlie a host of the python's postprandial responses. One of the nonadrenergic-noncholinergic factors stimulating the digesting python's heart is histamine, probably released by cardiac mast cells (Skovgaard and Wang 2008; Skovgaard et al. 2009). The importance of luminal signals—in particular, amino acids and peptides—in triggering intestinal response is revealed by the lack of cellular hypertrophy in segments of intestine that had been surgically isolated from contact with luminal nutrients while still retaining intact neural and vascular supply (Secor et al. 2000b, 2002).

This brief description of the postprandial responses of intermittently feeding snakes highlights the integration of their respiratory, cardiovascular, and gastrointestinal performances. Possessing unprecedented magnitudes of morphological and functional changes with feeding and fasting, these snakes, and other intermittently feeding vertebrates, provide an excellent opportunity to explore further the integration of organ performance, regulatory signals, and the underlying cellular mechanisms of tissue response (Secor 2008). In light of the breadth of this symposium, it would also be interesting to examine how the structure of the gut microbial community in these snakes is correlated with intestinal performance and affected by long episodes of digestive quiescence interspersed by large meals.

Dietary Specialization

The range of diets observed among animals in nature has been a topic of interest among biologists for centuries. Some omnivores such as grizzly bears consume a tremendous array of both animal and plant material. In contrast, other species concentrate on very narrow categories of acceptable diets, including pandas and koalas, which specialize on bamboo and eucalypt leaves, respectively. The evolution and ecology of these generalist and specialist dietary preferences has been a topic of considerable study by ecologists (Futuyma and Moreno 1988; Jaenike 1990; van Tienderen 1991; Wilson and Yoshimura 1994; Abrams and Schmitz 1999). Explanatory models are usually some variant of optimal foraging theory, predicting that organisms attempt to maximize energy intake while taking into account resource availability, time, and spatial constraints. Rarely, however, are actual energy gains measured experimentally or compared among specialist and generalist taxa. From a physiological point of view, we do not understand very well the digestive and energetic bases and consequences of dietary specialization.

Most of the thinking about dietary specialization assumes that there are trade-offs inherent in energy extraction ability related to dietary breadth (Futuyma and Moreno 1988; Fry 1996; McPeek 1996). Specialists are traditionally thought to be more efficient when feeding on a few dietary items. Generalists, in contrast, are regarded as using a wide variety of food with lower overall efficiency, in effect being jacks-of-all-trades and masters of none. These reciprocal patterns (specialization, high efficiency, narrow niche; generality, low efficiency, broad niche) are considered to be trade-offs, in which neither category is able to do everything well. While such trade-offs are widely expected, they remain to be empirically verified in any particular instance. Trade-offs may, for instance, be a general evolutionary correlate but may not occur in every individual case (Bennett and Lenski 2007).

At ICCPB4, Bennett and coworkers examined presumptive trade-offs in the digestive energetics in a group of closely related taxa of snakes (Britt et al. 2006, 2008; Bennett and Britt 2008). The garter snakes (genus *Thamnophis*) of western North America have some species or populations that are recognized as generalists and some that are extreme specialists (Arnold 1977, 1981; Drummond and Burghart 1983). For instance, *Thamnophis elegans elegans* eats fish, amphibians, leeches, and rodents, while the subspecies *T. elegans terrestris* eats only slugs (*Ariolimax* spp.). *Thamnophis ordinoides* also specializes on slugs, and *Thamnophis couchii* eats mainly fish. Slugs are difficult to consume and digest and have low energy content compared with fish or other prey items. The trade-off prediction suggests that the slug specialists should have become more efficient at extracting energy from slugs but lost their ancestral efficiency to digest fish and other generalist prey items. In fact, the first prediction is true: the slug specialists were found to be more efficient at processing slugs. Their assimilation efficiencies ((ingested energy − egested energy)/ingested energy) are around 70%, versus 55% for the other taxa, and their net

assimilation efficiencies ((ingested energy − egested energy − SDA)/ingested energy) are about 55%, versus 40%. The second part of the trade-off assumption, however, was not supported. All groups are equally good at obtaining energy from fish: assimilation efficiencies averaged 80% and net assimilation efficiencies averaged 70%. These results indicate that specialists may improve their ability to feed on novel prey without necessarily sacrificing their ability to feed on ancestral or more generalized prey items. This lack of parallelism in regard to dietary efficiency trade-offs may facilitate adaptation for feeding on locally available resources without jeopardizing the ability to continue to exploit other food sources.

Body size (and, hence, gut volume) may also affect dietary generality or specialization, as reported in studies of digestive capacity and immediate spare capacity in nectar-specialist birds (McWhorter and Martínez del Rio 2000; Martínez del Rio et al. 2001). 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 of their 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 by simply integrating maximum hydrolysis rates (V_{max}) along the entire length of the intestine (McWhorter and Martínez del Rio 2000). For example, total capacity to hydrolyze sucrose in 3-g broad-tailed hummingbirds (*Selasphorus platycercus*) estimated by integrating maximum sucrose hydrolysis rates indicated that capacity was about 120% higher than the observed rates of sucrose intake and digestion, implying that the immediate digestive spare capacity was quite high. By using a more sophisticated mathematical model of the gut as a plug-flow chemical reactor that included digestive efficiency as a factor (essentially a constraint regarding osmolyte and water loss; Jumars and Martínez del Rio 1999), McWhorter and Martínez del Rio (2000) calculated a more physiologically realistic immediate digestive capacity that was only ∼20% higher than observed rates of sucrose assimilation, closely matching digestive performance when birds were forced to feed at maximal rates using acute low ambient temperature challenges. At ICCPB4, McWhorter and coworkers reported results of a study designed to test digestive safety margins in passerine Australasian honeyeaters (Meliphagidae), varying in body size and degree of nectar specialization (T. J. McWhorter and P. A. Fleming, unpublished data). In the honeyeaters, digestive spare capacity relative to assimilated nutrient (sucrose) load increased with increasing body size, from ∼38% in the 10-g brown honeyeater (*Lichmera indistincta*) to ∼73% in the 23-g New Holland honeyeater (*Phylidonyris novaehollandiae*) and ∼144% in the 120-g red wattlebird (*Anthochaera carunculata*). In addition to being the largest species, the red wattlebird is also thought to be the least specialized, including more insects in its diet than the other species. In spite of this greater dietary breadth, it is no less efficient at digesting sucrose than the smaller, more specialized honeyeaters (all are >99% efficient). These results

suggest that there is neither a trade-off in digestive efficiency nor spare capacity with increasing diet breadth in honeyeaters. The pattern of increasing immediate digestive spare capacity with increasing body size in nectar-specialist birds is probably more closely linked to mass-specific metabolic rate than to diet specialization.

Conclusion

Comparative studies of the digestive system of vertebrates were founded on anatomical comparisons that were related to functions described in domesticated species of fish, birds, reptiles, and mammals (Van Soest 1994; Stevens and Hume 1995; Klasing 1998). Measures of digestive function in captive and wild vertebrates have increased progressively over the past two decades as more investigators have turned to nutritional studies to examine the effects of ecological changes (Karasov and Hume 1997; Hume 1999; Clements and Raubenheimer 2006) on individual animals that ultimately affect the growth of populations and the diversity of species (Karasov and Martínez del Rio 2007; Barboza et al. 2009). Recent expansions in the field have also been stimulated by new tools in molecular biology to examine the diversity of microbial symbionts and their enzymes, cell culture, and imaging technology to examine intestinal responses during a meal, and phylogenetic methods that have improved our ability to resolve differences between species and populations. Comparative physiologists continue to examine the digestive system because digestive processes are integrated with so many other systems, including thermoregulation, blood circulation, acid-base regulation, immunity, and reproduction.

Acknowledgments

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