Interplay between Physiology and Ecology in Digestion

William H. Karasov; Jared M. Diamond

BioScience, Vol. 38, No. 9. (Oct., 1988), pp. 602-611.

Stable URL:
http://links.jstor.org/sici?sici=0006-3568%28198810%2938%3A9%3C602%3AIBPAEI%3E2.0.CO%3B2-Z

BioScience is currently published by American Institute of Biological Sciences.

Interplay between Physiology and Ecology in Digestion

Intestinal nutrient transporters vary within and between species according to diet

William H. Karasov and Jared M. Diamond

Twenty years ago one of us (JMD), who then described himself professionally as a physiologist, went for a bird walk with ecologists Martin Cody and the late Robert MacArthur. Near a stream they saw a Black Phoebe, a species of flycatcher confined to the vicinity of water. To MacArthur's question, "Why do you suppose the Black Phoebe lives only near water?", Diamond and Cody gave opposite dogmatic responses. Diamond insisted, "There must be physiological reasons, like low renal concentrating ability resulting in high water requirements. Physiological factors often determine an animal's ecology." Cody replied equally firmly, "Nonsense. Natural selection makes an animal's physiology adapt to the animal's ecological niche, so that physiology provides nothing more than proximate causes. The ultimate causes must be ecological ones, like food availability near streams or else competition with flycatcher species of drier habitats."

Today, having outgrown the dogmatism of youth, we describe ourselves as both physiologists and ecologists and view the Black-Phoebe problem as more complex. We have ceased debating whether one of two mutually exclusive views is correct:

Whether physiology constrains ecology, or vice versa, depends on the time required for adaptation

Does physiology constrain ecology, or vice versa? Instead, we accept that there is a complex interplay between physiology and ecology, involving variously flexible constraints upon adaptation at various time scales. Yes, physiological systems may adapt to ecological variables, on time scales ranging from the millisecond of a nerve impulse to the millenia of evolutionary time. Conversely, physiological systems that are rigid or slow to adapt may limit a species' behavior, hence its ecological niche and its evolution. The time scale of physiological adaptation is thus a crucial issue. These so-called inherent constraints are emerging as a difficult major problem in ecology, evolutionary biology, and sociobiology (cf. Rubenstein and Wrangham 1986).

For many reasons, intestinal absorption of nutrients provides a good testing ground to study this interplay between physiology and ecology. The physiological mechanisms of nutrient absorption by vertebrate intestine are well understood (Johnson 1987). Nutrient absorption is of obvious ecological significance: indeed, ecological models often begin by assuming that natural selection acts to maximize the acquisition of net metabolizable energy, among other things (Krebs and

Telfair's skink (*Leiolopisma telfairii*) is an omnivore, frequently eating seabird eggs, such as this shearwater egg. This skink is an endangered species endemic to Round Island in the Indian Ocean. Photo: Stanley A. Temple, University of Wisconsin.

William H. Karasov is an assistant professor in the Department of Wildlife Ecology, University of Wisconsin, Madison 53706. Jared M. Diamond is a professor in the Department of Physiology, University of California at Los Angeles School of Medicine, Los Angeles 90024.

Davies 1984). In recent years several studies have examined the regulation of intestinal nutrient transport mechanisms within an ecological and evolutionary context (Diamond and Buddington 1987, Karasov 1987, Karasov and Diamond 1983a, Karasov et al. 1985a). These studies are proving relevant to questions about limiting factors for foraging behavior, ontogenetic development, and evolutionary adaptation.

This article focuses on one step in absorption: uptake of sugars and amino acids across the cell membrane separating the lumen of vertebrate intestine from the cytoplasm of the intestinal absorptive cells. We begin by summarizing information on the transport mechanisms, a convenient method for studying them, and their relation to other steps in absorption. We then survey glucose uptake rates among vertebrate species of different natural diets, to test our a priori expectation that glucose uptake rates vary interspecifically with the carbohydrate content of the natural diet (Karasov and Diamond 1983a).

After dissecting the contributions of phenotypic and genotypic adaptation to this interspecific variation, we next ask whether uptake rates vary developmentally within an individual animal's lifetime as the animal traverses the age-related sequence of diets characteristic of its species. Finally, we discuss how these findings about nutrient absorption exemplify the interplay of physiological and ecological constraints on different time scales. We do not address here the related problem of the intestinal adaptations that enable endotherms to sustain metabolic and feeding rates an order of magnitude higher than those of ectotherms (Karasov 1987, Karasov and Diamond 1985, Karasov et al. 1985a).

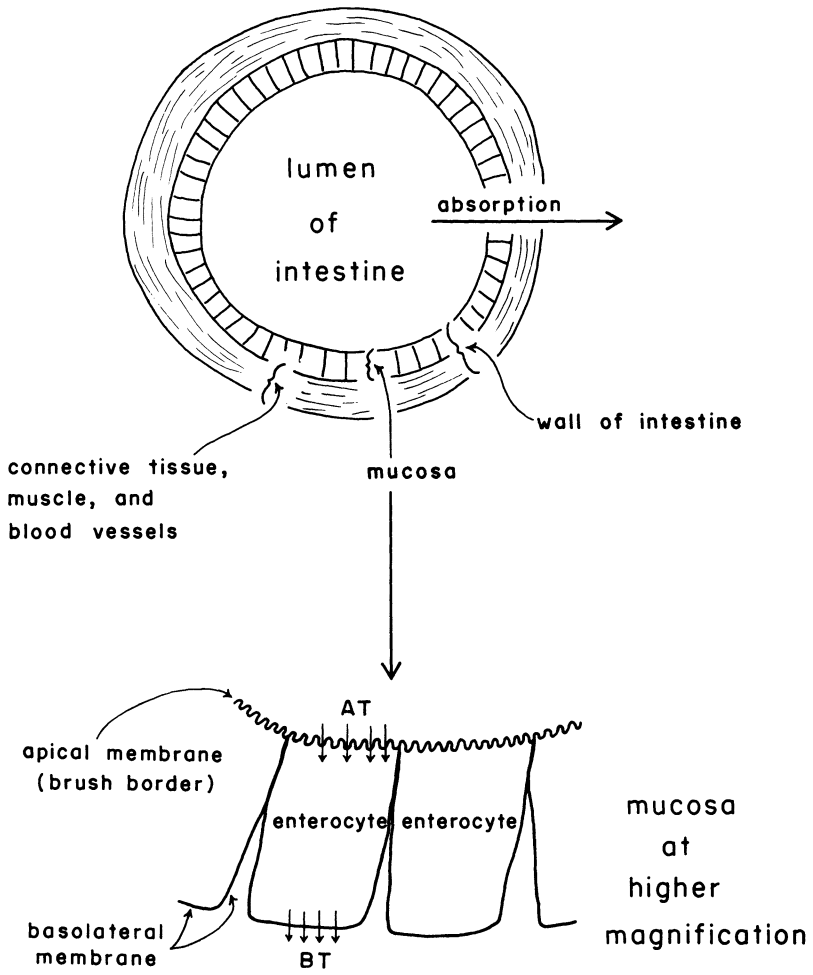

Figure 1. Schematic cross-section of vertebrate intestine. The lumen is lined by a single layer of absorptive cells, the mucosa, supported on the outside by smooth muscle and connective tissue containing blood vessels. The cell membrane at the surface of the mucosal cells (enterocytes) facing the lumen is called the apical membrane, while the membrane facing the connective tissue is called the basolateral membrane. The apical membrane is also termed the brush border because of its densely packed microvilli. Nutrient absorption involves transport proteins (apical and basolateral transporters: AT and BT in the illustration) that convey specific nutrient molecules across each of these membranes.

Intestinal transporters

Proteins and complex carbohydrates in food are hydrolysed by the digestive enzymes of saliva, pancreatic juice, intestinal secretions, and intestinal cell membranes to yield small peptides, free amino acids, and monosaccharide sugars (see Johnson 1987 for a book-length treatment of digestive physiology). These smaller molecules are then absorbed in the proximal or small intestine (but also in the distal intestine, colon, and pyloric or distal caeca in some species).

Measurements of the competition among different solutes for absorption have helped define many separate absorptive mechanisms, which are called transporters. For example, there is at least one transporter for aldohexoses like glucose, another for ketohexoses like fructose; one transporter for basic amino acids like lysine, another for acidic amino acids like aspartate, another for neutral amino acids like leucine, and still another for the imino acid proline. There are also transporters for water-soluble vitamins, minerals, and some fatty acids (Johnson 1987).

Of the intestinal nutrient transporters, only that for glucose has been characterized at the molecular level (Hediger et al. 1987), but the others are assumed to be membrane proteins by analogy with the glucose transporter and with the many identified absorptive mechanisms of other eukaryotic cells and bacteria. Most of the transporters are active (i.e., they can absorb substrate against a concentration gradient by means of energy provided by a sodium concentration gradient maintained by ATP).

The intestinal lumen is lined by a

single uninterrupted sheet (the mucosa) of cells (called enterocytes). Thus, solutes absorbed from the intestinal lumen into the bloodstream must actually traverse two cell membranes in series: the apical or brush-border membrane, which separates the intestinal lumen from the enterocytes' cytoplasm, and the basolateral membrane, which separates the enterocytes' cytoplasm from the connective tissue, smooth muscle, and blood vessels forming the outside of the intestine (Figure 1). Each of these two membranes has its own set of transporters for sugars and amino acids. This article considers only the transporters in the apical membrane, because far more is known about them than about the basolateral transporters and because the apical step is the one against a concentration gradient. However, the basolateral step is also subject to adaptive regulation (Maenz and Cheeseman 1986).

Each of the various experimental methods for measuring intestinal transport poses technical problems. Hence we use a single experimental method for species comparisons. In this simple in vitro technique, termed the everted sleeve method (Karasov and Diamond 1983b), we place a one-centimeter length of everted intestine mounted over a solid rod in a physiological salt solution appropriate for the particular species and which contains a ^{14}C- or ^{3}H-labeled nutrient. We can then determine the quantity of nutrient taken up across the apical membrane (facing the outside of the everted sleeve) into the enterocytes by counting tissue radioactivity at the end of the incubation (usually lasting one to four minutes) and correcting for radioactivity in the adherent fluid.

The concentration dependence of nutrient uptake generally follows the saturable kinetics typical of carrier-mediated transport. For species comparisons we use a nutrient concentration, usually 50 mM, sufficient to saturate the carrier. The everted sleeve method has been readily applied to the intestines of all 40 of the small-bodied species that we have examined. These species (ranging from sturgeon, snakes, and tadpoles to hummingbirds and mink) include all five higher vertebrate classes.

Like uptake across the apical membrane, many other steps in nutrient absorption are regulated, are ecologically important, and differ among species. These steps include transport across the basolateral membrane, hydrolysis by enzymes in the apical membrane or in pancreatic juice, and metabolism by the intestinal mucosa or liver or gastrointestinal symbionts. Many anatomical features of the gastrointestinal tract also vary interspecifically (such as gut length and presence or absence of diverticula) and are ecologically important.

Thus, we do not claim to be measuring the parameter in nutrient absorption with the greatest ecological significance, but just one of many physiological and anatomical parameters. Eventually, when further information becomes available, it may prove more illuminating to examine the ecological significance of a whole suite of physiological and anatomical adaptations for nutrient use, rather than of a single step alone. It may also prove illuminating to study not only interspecies differences in uptake, as we have done to date, but also geographic differences within a species and individual differences within a population, as illustrated by Arnold's (1981a, b) studies of feeding by garter snakes.

Natural diet and intestinal nutrient transporters

Modern vertebrates differ greatly in diet. Frugivores and nectarivores consume diets high in carbohydrates, carnivores consume a high-protein diet, and planktivorous seabirds consume a high-lipid diet. Do activities of apical nutrient transporters vary interspecifically according to the dietary levels of their substrates?

As one example, consider three bird species with different natural diets: nectarivorous hummingbirds *(Selasphorus rufus)*, omnivorous chickens *(Gallus gallus)*, and carnivorous shrikes *(Lanius ludovicianus)* (Karasov et al. 1986). We fed, for at least one week, sugar water to the hummingbirds, a commercial chicken feed to the chickens, and raw pieces of chicken to the shrikes. In each species we then measured intestinal uptake of the sugar D-glucose and the amino acid L-proline at a concentration of 25 mM or 50 mM. L-proline was

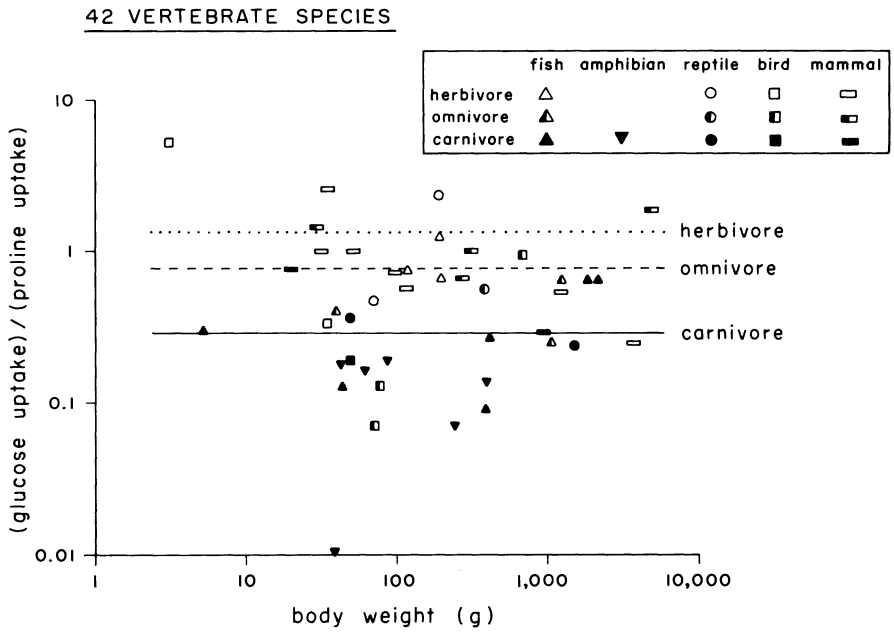

Figure 2. Relative rates of sugar and amino acid transport in herbivorous, omnivorous, and carnivorous species of all five higher vertebrate classes. Each species was eating its natural diet or one of nutrient composition similar to the natural diet. The ordinate is the ratio of the intestine's total uptake capacity for the sugar glucose to its uptake capacity for the amino acid proline. Note that this ratio is highest for herbivores, intermediate for omnivores, and lowest for carnivores, but it is independent of body weight. There is no significant dependence of these ratios on body mass, so the horizontal lines depict average values of the ratio for each trophic group. After Diamond and Buddington 1987, with additional data.

chosen as a general probe for amino acid transport because it is transported by at least two of the amino acid transporters (Karasov 1988).

Proline uptake (per cm^2 of nominal surface area [neglecting villi, ridges, and microvilli] or per mg weight of intestine) proved similar among the three species. But glucose uptake in the hummingbird was 15 times higher than that in the chicken and 50 times higher than that in the shrike.

As an alternative comparison, we calculated for each species the glucose:proline (G/P) ratio, defined as the intestine's summed uptake capacity for glucose divided by that for proline, where summed uptake capacity represents the rate of absorption (at a substrate concentration sufficient to saturate the transporters) summed over the entire length of the intestine. This ratio has several virtues in comparative studies of nutrient absorption: it bypasses entirely the difficult question of to what measure of intestinal tissue the nutrient uptake rates should be normalized (e.g., whether to intestinal length, area, mass, or protein content); it permits comparisons between species of different body mass because, although summed uptake rates increase with body mass, they do so with a similar scaling for both glucose and proline (Karasov 1987); and it permits comparisons between endotherms and ectotherms, which tend to differ greatly from each other in their summed uptake rates, but the differences are in the same proportion for glucose and proline (Karasov 1987). In our comparison of the three bird species, this G/P ratio was 5.25 for the hummingbird, 0.95 for the chicken, and 0.19 for the shrike.

Figure 2 shows that G/P ratios of all 40 vertebrate species studied (Table 1) fit the same trend as the three-species avian comparison. The measurements depicted in Figure 2 were made on animals being maintained in the laboratory on their natural diets or on artificial diets simulating the natural ones. For example, we fed crickets (high in protein, low in carbohydrate) to the carnivorous reptiles, but fed alfalfa pellets and lettuce (low in protein, high in carbohydrate) to the herbivorous reptiles. The G/P ratio is highest for herbivores (mean ± S.E.M.: 1.27 ± 0.36, n = 14 species), somewhat lower for omnivores (0.73 ± 0.17, n = 11 species), and lowest for carnivores (0.33 ± 0.07, n = 16 species). The carnivore value is significantly lower than that for omnivores ($P < 0.025$) or for herbivores ($P < 0.01$). Thus, activity of the brush-border glucose transporter varies interspecifically with the carbohydrate content of the natural diet.

Neither this overall comparison, nor that among the three bird species, involved closely related species with different dietary habits. Hence both comparisons might be confounded by phylogeny (Ridley 1983). Could high G/P ratios of herbivores be merely a legacy of common descent of the particular herbivores studied, rather than a general characteristic of herbivores? For example, in several cases Table 1 contains data for two or three species that belong to the same phylogenetic line (e.g., two carp species, three toad species, two carnivorous snakes, and two lagomorphs) and that yield similar G/P values. These multiple values could bias our statistical comparisons if the transport characteristics evolved prior to speciation (Ridley 1983). However, when we base comparisons instead on a single mean G/P ratio for each such set of species, the difference in G/P ratio between carnivores and herbivores/omnivores still proves significant ($p < 0.05$) despite the lower sample size and hence fewer degrees of freedom.

Another solution to the confounding effect of phylogeny is to make the comparison in many independent phylogenetic lines (Ridley 1983). Thus, it is reassuring to note that the trend of higher G/P ratio in herbivore/omnivores than carnivores holds true within each vertebrate class that we studied (except for amphibians, where it could not be examined because all are carnivores as adults). The G/P ratio does not vary significantly with body mass, nor does it differ significantly among herbivores (or omnivores, or carnivores) from different vertebrate classes, nor does it differ significantly among endotherms (mammals and birds) and ectotherms (reptiles, amphibia, and fish).

As in the hummingbird/chicken/shrike comparison, species differences in the G/P ratio mainly reflect a decline in glucose uptake from herbivores to omnivores to carnivores; proline uptake is much less dependent on trophic habits. Species differences in glucose uptake rates per milligram of intestinal tissue in turn largely reflect species differences in the number of copies of the glucose transporter, as measured by kinetic analysis of transporter binding of phlorizin (Ferraris et al. in press). (Phlorizin is a selective, membrane-impermeant, competitive inhibitor of active D-glucose transport in the brush border). We have as yet no evidence of significant qualitative differences among vertebrates in their intestinal glucose transporters (Karasov 1988).

Why should natural selection have led to a much closer correlation between trophic habits and sugar transporter number than between trophic habits and amino acid transporter number? We suggest the following explanation. Amino acids are essential nutrients that all species, regardless of their trophic habits, require for growth and maintenance; carnivores merely consume some additional amino acids for calories. Thus, dietary protein requirements and intestinal amino acid transporter activities may be only modestly dependent on trophic habits. In contrast, sugars furnish nothing more than calories; they are not nutritionally essential, and the actual carbohydrate content of the diet of strict carnivores is very low. Yet synthesis and maintenance of the molecular machinery to absorb and metabolize carbohydrate constitute an ongoing biosynthetic expense that can be justified only by the payoff in calories. That molecular machinery becomes useless on a low-carbohydrate diet and tends to be reduced in carnivores, just as natural selection tends to eliminate functional eyes in cave animals and functional wings in birds of remote predator-free islands. The detailed mechanism by which such useless structures are lost poses an interesting unsolved problem (Diamond 1986, Dykhuizen 1978).

Phenotypic versus genotypic adaptations

Levels of many enzymes vary reversibly with substrate levels over the course of a few minutes to a few days, as a result of enzyme synthesis being

Table 1. Summed uptake capacity for D-glucose and L-proline in 40 species of vertebrates.

Species*	Species diet†	Body mass (g)	Summoned uptake rate (μmole/min) glucose	proline	Reference
FISH					
prickleback‡ (*Cebidichthys violaceus*)	c	5.1	0.04	0.12	Buddington et al. 1987
prickleback‡ (*C. violaceus*)	o	39.1	0.25	0.62	Buddington et al. 1987
striped bass‡ (*Morone saxatilis*)	c	43	0.06	0.43	Buddington et al. 1987
striped bass‡ (*M. saxatilis*)	c	1871	2.43	3.74	Buddington and Diamond 1987
tilapia (*Sarotherodon mossambicus*)	h	120	2.11	2.82	Buddington et al. 1987
common carp (*Cyprinus carpio*)	h	193	5.64	4.0	Buddington et al. 1987
grass carp (*Ctenopharyngodon idella*)	h	200	2.00	2.78	Buddington et al. 1987
rainbow trout (*Salmo gairdneri*)	c	391	0.70	8.17	Buddington and Diamond 1987
largemouth bass (*Micropterus salmoides*)	c	401	0.92	3.41	Buddington and Diamond 1987
white sturgeon (*Acipenser transmontanus*)	o	1050	2.10	7.67	Buddington et al. 1987
channel catfish (*Ictalus punctatus*)	o	1260	7.31	11.72	Buddington et al. 1987
cod (*Gadus morhua*)	c	2097	2.73	4.19	Buddington and Diamond 1987
AMPHIBIANS					
African clawed frog (*Xenopus laevis*)	c	43.5	0.05	0.24	Buddington§
American toad (*Bufo americanus*)	c	39	0.01	0.35	Buddington§
western toad (*Bufo boreus*)	c	62	0.085	0.546	Buddington§
Woodhouse's toad (*Bufo woodhousei*)	c	87	0.126	0.675	Buddington§
mudpuppy (*Necturus maculosus*)	c	250	0.11	1.575	Buddington§
bullfrog (*Rana catesbeiana*)	c	400	1.37	9.57	Karasov et al. 1985c
REPTILES					
western garter snake (*Thamnophis elegans*)	c	50	7.13	19.35	Karasov‖
desert iguana (*Dipsosaurus dorsalis*)	h	71	2.1	4.4	Karasov et al. 1985a
chuckwalla (*Sauromalus obesus*)	h	191	8.5	3.6	Karasov et al. 1985a
box turtle (*Terrapene carolina*)	o	384	6.0	10.6	Karasov et al. 1985a
gopher snake (*Pituophis melanoleucus*)	c	1540	5.69	23.83	Buddington§
BIRDS					
rufous hummingbird (*Selasphorus rufus*)	n	3.2	1.26	0.24	Karasov et al. 1986
cedar waxwing (*Bombycilla cedrorum*)	f	35	2.1	6.2	Martinez del Rio and Karasov#
shrike (*Lanius ludovicianus*)	c	48	0.69	3.53	Karasov et al. 1986
starling (*Sturnus vulgaris*)	o	71	0.60	9.0	Levey and Karasov**
robin (*Turdus migratorius*)	o	79	1.49	11.09	Levey and Karasov**
chicken (*Gallus gallus*)	o	700	7.91	8.32	Karasov et al. 1986
MAMMALS					
mole shrew (*Blarina brevicauda*)	c	20	9.62	12.71	Karasov‖
laboratory mouse (*Mus musculus*)	o	30	22.0	15.3	Karasov et al. 1983
prairie vole (*Microtus ochragaster*)	h	32	8.47	8.44	Karasov‖
kangaroo rat (*Dipodomys merriami*)	g	35	8.0	3.1	Karasov et al. 1985c
fruit bat (*Artibeus jamaicensis*)	f	51	10.7	10.1	Karasov et al. 1985c
golden hamster (*Mesocricetus auratus*)	h	102	12.6	17.8	Karasov et al. 1985a
woodrat (*Neotoma lepida*)	h	121	14.5	25.5	Karasov et al. 1985a
laboratory rat (*Rattus norvegicus*)	o	276	22.7	34.1	Karasov and Debnam 1987
Belding's gr. squirrel (*Spermophilus beldingi*)	o	318	27.5	27.6	Karasov et al. 1985c
mink (*Mustela vison*)	c	950	39.2	133.7	Buddington§
snowshoe hare (*Lepus americanus*)	h	1262	31.9	57.9	Karasov‖
rabbit (*Oryctolagus cuniculus*)	h	3630	32.8	137	Buddington§
monkey (*Cercopithecus aethiops*)	o	5000	136	70	Karasov et al. 1985c

*Measurements on amphibians and fish were made at 20° C and all measurements on reptiles, birds, and mammals were made at 37° C.
†c = carnivore or insectivore; o = omnivore; h = herbivore; n = nectarivore; g = granivore; f = frugivore.
‡Different size classes of these species have different diets and proved to have significantly different uptakes (Buddington et al. 1987).
§R. K. Buddington, 1987. Unpublished data. Department of Physiology, University of California, Los Angeles.
‖W. H. Karasov, 1988. Unpublished data.
#C. Martinez del Rio and W. H. Karasov, 1987. Unpublished data. Department of Zoology, University of Florida, Gainesville.
**D. J. Levey and W. H. Karasov, 1987. Unpublished data. Department of Zoology, University of Florida, Gainesville.

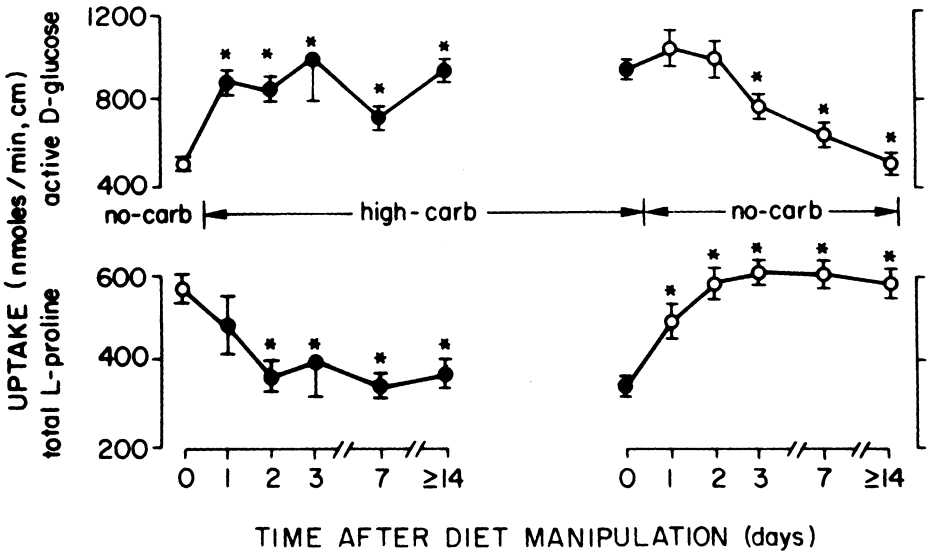

Figure 3. Time course for effect of change in dietary substrate levels on active D-glucose uptake and L-proline uptake at 50 mM in mouse jejunum. Left half of figure: mice switched from no-carbohydrate (no-carb) high-protein diet (open circles) to high-carbohydrate (high-carb) low-protein diet (solid circles) at time t = 0; subsequent points give uptake rates for glucose (top) and proline (bottom) after the indicated number of days on the high-carb diet. Right half: a separate experiment in which mice were switched from high-carb diet to no-carb diet at t = 0; subsequent points give uptake rates after the indicated number of days on the no-carb diet. Asterisks indicate uptake values that differ significantly from the corresponding t = 0 value. Note that glucose uptake and proline uptake increase reversibly with dietary carbohydrate and protein levels, respectively. From Karasov et al. 1983.

induced or repressed by an enzyme's substrate. For example, the Russian biologist Ivan Pavlov observed a century ago that high-carbohydrate or high-protein diets led to high pancreatic levels of the starch-digesting enzyme amylase or the protein-digesting enzyme trypsin, respectively. If the same were true of intestinal nutrient transporters, a high-carbohydrate or high-protein diet might induce glucose or proline transporters. Do the species differences of Figure 2 depend at least in part on phenotypic regulation of transporter activities by substrate levels in the differing diets that the animals were consuming at the time of sacrifice?

Figure 3 demonstrates that phenotypic regulation of glucose and proline transporters does occur in mice. When mice are switched from a high-protein carbohydrate-free diet to a low-protein high-carbohydrate diet, glucose transporter activity rises and proline transporter activity declines. The reverse diet switch produces opposite changes in the transporters. The changes in glucose transporter activity are due to changes in the number of functional copies of the transporter (Ferraris and Diamond 1986).

Such experiments have been performed for 17 different intestinal nutrient transporters, and all but two prove to be regulated by dietary substrate levels, though the regulatory patterns vary among the transporters. Two sugar transporters (those for aldohexoses and ketohexoses) and two amino acid transporters (those for imino acids and acidic amino acids) are induced by their substrates; three vitamin transporters (those for biotin, thiamine, and ascorbic acid) and five mineral transporters (those for iron, calcium, phosphate, zinc, and possibly copper) are repressed by their substrates; activities of two amino acid transporters (those for neutral and basic amino acids) and perhaps one peptide transporter vary nonmonotonically with dietary substrate levels; and two vitamin transporters (those for pantothenate and choline) have activities apparently independent of substrate levels. These transporter differences in regulatory patterns make functional sense in terms of whether a transporter's substrate is nutritionally essential, is toxic at high levels, or yields calories (Diamond and Karasov 1987, Ferraris and Diamond in press).

Might such phenotypic regulation account entirely for the measured species differences? If herbivores, omnivores, and carnivores could be persuaded at least temporarily to consume the same ration, would the species differences of Figure 2 disappear? We do not know the answer for mammals, because of the difficulty of finding a ration equally acceptable to cows and tigers. However, the experiment can be performed for fish, because some fish species with different natural diets will accept manufactured diets that in nutrient composition are quite unlike their natural diets.

Figure 4 depicts results of an experiment in which three carnivorous, two omnivorous, and four herbivorous fish species were maintained in the laboratory for more than eight weeks on the same ration (commercial trout feed), which contains approximately 45% protein and 25% digestible carbohydrate (Buddington et al. 1987). Hence any contribution of phenotypic regulation (in response to differing rations) to species differences in transporter activities was eliminated, and any remaining differences must be hard-wired genetically. We found that the G/P ratio did decrease from herbivores (0.63 ± 0.16) to omnivores (0.36 ± 0.15) to carnivores (0.12 ± 0.04), just as it did in Figure 2, though to a smaller extent. This trend in the ratios is mainly due to species differences in glucose uptake rather than in proline uptake.

This demonstration of apparent genetic hard-wiring is subject to the caveat that transporter activity in adulthood must not have been fixed irreversibly by an animal's ration early in life. But we think this unlikely, because an explicit search in mice for such critical-period programming of intestinal transport failed to detect such an effect (Karasov et al. 1985b).

Both phenotypic and genotypic effects therefore contribute to the results of Figure 2. Herbivores are genetically programmed to maintain higher glucose transporter activities than carnivores, even in the absence of any immediate difference in diet

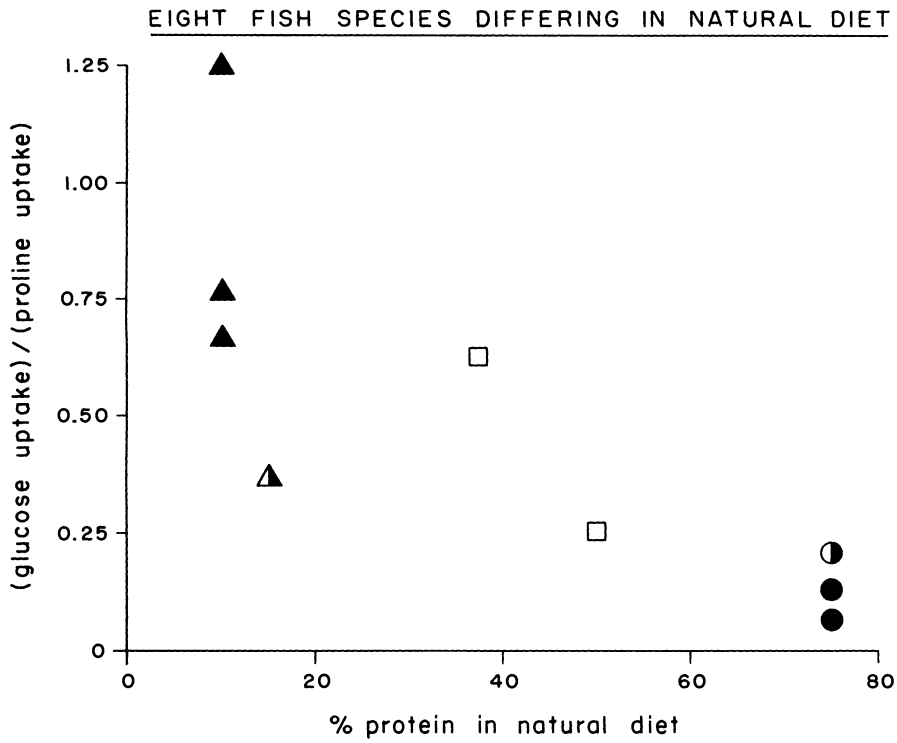

Figure 4. The intestine's summed uptake capacity for glucose divided by that for proline (ordinate), as a function of the percentage of protein in the natural diet (abscissa), for eight fish species. ●, carnivores; □, omnivores; ▲, herbivores; ◑ and ◭, a species that is carnivorous while small (◑) but is herbivorous when large (◭). Note that the ratio of glucose to proline uptake decreases with the proportion of protein in the natural diet, even though all eight species were studied while eating the same artificial diet. Thus, the differences in the glucose/proline uptake ratio depicted in this figure must be genetic adaptations to the natural diet and cannot be reversible phenotypic responses to the immediate diet. After Buddington et al. 1987.

(Figure 4). In addition, herbivores normally consume a diet with much higher carbohydrate levels than the normal diet of carnivores, and this dietary carbohydrate induces a further increase in the herbivores' glucose transporter activity (Figure 3).

Interestingly, such induction has been demonstrated in all three herbivorous and omnivorous species examined—laboratory mouse (Diamond and Karasov 1984), laboratory rat (Ginsburg and Heggeness 1968), and common carp (Buddington 1987)—and in one carnivore (mink) belonging to a subfamily that includes omnivorous species (Mustelinae). Induction has not been demonstrated in four strict carnivores belonging to strictly carnivorous families—rainbow trout, two species of frogs, and the domestic cat.[1] Hence carnivorous families not only have low basal activity levels of the glucose transporter but also lack the regulatory machinery that would enable them to increase transporter activity on a high-carbohydrate diet.

[1]R. K. Buddington and J. W. Chen. 1987. Unpublished observations. University of California, Los Angeles.

Ontogenetic development

Our discussion of the relation between natural diet and nutrient transporter activities was based on adult animals. But individuals of many vertebrate species undergo dramatic changes in diet as they develop. Bullfrogs change from herbivorous tadpoles into carnivorous adult frogs; many fish species shift from carnivory towards omnivory or herbivory as they grow; and infant mammals subsist on a diet of milk before switching to an adult diet of very different composition. These developmental changes in diet pose three related questions concerning intestinal nutrient transporter activities: Do transporter activities also change developmentally? If so, what is the functional significance of the changes? What are the immediate signals causing the changes?

Transporter activity has been followed from birth, hatching, or early in life until adulthood in nine species chosen from four of the five higher vertebrate classes: channel catfish and monkeyface prickleback (Buddington et al. 1987); sheep (Scharrer 1976); and bullfrogs, chickens, laboratory rats, rabbits, cats, and desert woodrats (see Buddington and Diamond in press). Transporter activities in all these species exhibit marked developmental changes, some of which are parallel to developmental changes in dietary inputs of their substrates. For example, in most suckling mammals the main dietary sugar is lactose (a disaccharide composed of glucose plus galactose), and fructose becomes dietarily significant (if at all) only after weaning. Not surprisingly, intestinal fructose transporter activity in rats and rabbits increases manyfold at the time of weaning. As another straightforward example, glucose and galactose transporter activity in sheep declines steeply around the time of weaning, after which ruminal bacteria ferment ingested carbohydrate with the result that little sugar reaches the intestine.

A rise in the G/P ratio has been observed during the development of at least four of the species studied so far. In parallel with this rise in the G/P ratio, the ratio of carbohydrate to proline also rises in the natural diets of three of these species: rats, which switch from high-protein milk to high-carbohydrate solid food, and catfish and pricklebacks, which shift from carnivory to omnivory or herbivory. Thus, one might at first see the functional significance of the changing G/P ratio as just another example of transporter activities matching the inputs of their dietary substrates.

But the G/P ratio also rises from tadpoles to bullfrogs, which experience the opposite dietary switch. (In this case the herbivorous tadpoles eat the high-carbohydrate diet, while the carnivorous frogs eat the high-protein diet.) Recall, however, that growing young animals generally have higher amino acid requirements than adults. Thus, the functional significance of

the initially low G/P ratio may be to provide the amino acids needed for growth rather than to match the diet. In future research, this hypothesis can be tested by examining whether the G/P ratio rises developmentally in most vertebrate species, regardless of how the diet changes with age.

One of the most interesting questions in developmental biology concerns the signals that cause each biological process to switch on or off at the appropriate time. These signals can either be external to the animal, such as light, or else hard-wired genetically within the animal, such as an intracellular timer or a preprogrammed release of a hormone. For example, are the changes in intestinal nutrient transport in mammals at the time of weaning triggered by the act of weaning itself (e.g., by the change in dietary solute input to the intestine), or are they instead controlled by a cellular timer, such that the transport changes would take place at the same age even if weaning were experimentally advanced or retarded?

Hard-wired control of nutrient transporters has been established for two fish species and one mammal (Buddington et al. 1987, Buddington and Diamond in press). As mentioned above, prickleback and catfish normally undergo a switch from carnivory towards herbivory and a rise in the G/P ratio as they develop. However, individuals of each species maintained in the laboratory on a constant diet still show a size-related rise in the G/P ratio (Figure 5).

Similarly, the fructose transporter is first present in rat intestine only after about three weeks of age, when weaning normally occurs and dietary fructose is first encountered. However, if infant rats are maintained instead on a fructose-free artificial milk until the age of six weeks, the fructose transporter is still present at three weeks. Thus, in these cases the developmental changes in transporters do not require the dietary change as a proximate signal. The animal is instead hard-wired genetically to be prepared for the dietary shifts normally encountered during its development.

Interplay between physiology and ecology

Let us now use these studies of intestinal nutrient transporters to illuminate the Black-Phoebe problem with which we began this article: Does physiology constrain ecology or vice versa? To anyone who has thought seriously about this problem, it should come as no surprise that the question proved inappropriately framed, demanding a simple answer to a complex problem. It is instructive instead to examine the various time scales on which intestinal nutrient processing adapts to a change in diet, since the speed of the physiological response determines whether the response is relevant to daily variations in an individual animal's foraging behavior, to an individual's growth and development, or only to a species' evolutionary potential.

At the fast extreme, nerve reflex arcs enable salivary secretions containing the starch-digesting enzyme amylase to be released within a few seconds of ingesting (or even just smelling) food. Within a few minutes of food arriving at the intestine, the hormones secretin and pancreozymin stimulate production of pancreatic juice containing many digestive enzymes. Within six hours of a rise in blood sugar concentrations, the activity of sugar transporters rises in the basolateral membranes of enterocytes (Csaky and Fischer 1981, Karasov and Debnam 1987, Maenz and Cheeseman 1986). One-half to three days after a rise or fall in dietary carbohydrate levels, glucose transporter activity rises or falls in the apical membranes of enterocytes (Diamond and Karasov 1984). One week or more is required for enterocytes to proliferate in response to the increased nutrient requirements and food intake that accompany pregnancy and lactation (Karasov and Diamond 1983a).

All these adaptive responses are reversible, occurring repeatedly in an individual's lifetime, but the hard-wired developmental changes in nutrient transporters around the time of weaning are irreversible, occurring only once in a lifetime. For nutrient transporters, there may also be other irreversible, once-in-a-lifetime, critical-period effects awaiting discovery (Karasov et al. 1985b), analogous to imprinting effects in animal behavior or the irreversible fixing of human sweat gland function by the thermal environment during infancy (Kuno 1956).

Finally, the slowest adaptations are genetic ones that depend on natural

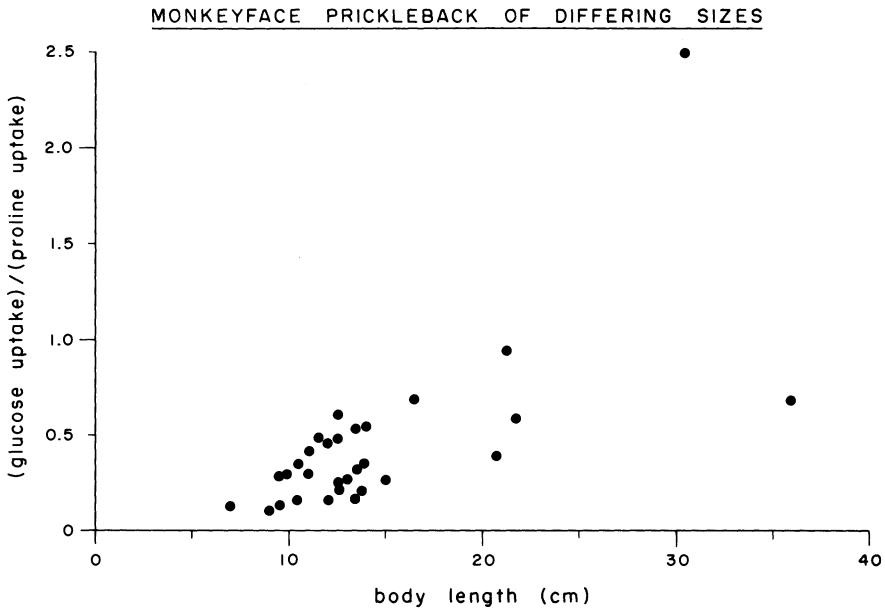

Figure 5. The intestine's summed uptake capacity for glucose divided by that for proline (ordinate), plotted against body length, for a fish species called the monkeyface prickleback. Large pricklebacks (herbivorous in the wild) have higher rates of glucose uptake relative to proline uptake than do small pricklebacks (carnivorous in the wild), even though both were studied while eating the same artificial diet in the laboratory. Thus, the intestine is hard-wired to shift the activities of its nutrient transporters as the fish grows, even in the absence of the dietary change that the fish would normally undergo in the wild. After Buddington et al. 1987.

selection and require many generations. These population adaptations include changes in allele frequencies as well as individual adaptations through new mutants or recombinants. From intestinal physiology, possible examples of genetic adaptations include species differences in glucose transporter activity correlated with natural diet but not with immediate diet (Figure 4), and species differences in presence or absence of the machinery for regulating glucose transporter activity.

Some of the consequences of these physiological adaptations for animal behavior and ecology are obvious. The species that we have examined possess nutrient transporters appropriate to their needs and natural diets: amino acid transporters in herbivores as well as in carnivores, higher sugar transporter activity in herbivores than in carnivores. Several species with variable natural diets are able to regulate glucose transporter activity according to dietary carbohydrate levels. As one distinguished comparative physiologist reflected on surveying his life's work, "Animals usually prove to be physiologically adapted to what they're doing!"

Less obvious are some cases in which physiological limitations constrain behavior and ecology. As one example, consider the consequences of glucose transporter activity for migratory hummingbirds (Karasov et al. 1986). Because of their small body size and energetically expensive mode of foraging by hovering, hummingbirds have mass-specific basal and active metabolic rates among the highest of any animal. Hummingbirds' main source of calories is nectar with high sugar concentrations (up to 2 M). During their southward migration through western North American mountains in autumn, possibly fatal bouts of freezing conditions may occur unpredictably. Therefore, hummingbirds are under extreme natural selection to migrate rapidly, and thus to ingest sugar and convert it to fat as quickly as possible.

This selection doubtless explains why hummingbird intestine has evolved the highest glucose transporter activity recorded for any vertebrate and why the nectar is processed so rapidly (transit time from mouth to vent is less than 15 minutes). It is disconcerting, however, to observe that hummingbirds spend only about 20% of their time feeding, 75% of their time sitting and apparently doing nothing (Ewald and Carpenter 1978, Hixon et al. 1983). The explanation proves to be that hummingbirds do not refill their crop until the previous nectar meal has been cleared from the crop into the intestine, but the crop emptying rate appears to be limited by the rate at which the intestine can absorb the high concentrations of glucose in nectar. As for why hummingbirds did not just evolve more intestinal sugar transporters, the fact that they have a record-high glucose transporter activity suggests that they may already have reached the attainable upper limit. Thus, hummingbird behavioral ecology is subject to a bottleneck imposed by digestive physiology, analogous to the more familiar bottleneck that the time required for fiber digestion imposes on cows and other ruminants.

As Krebs and Harvey (1986) commented in connection with this hummingbird example, "Optimization processes evolve under certain constraints, and rates of digestion may provide a more widespread constraint to activity patterns than previously thought." Krebs and Harvey (1986) and Hanski (1984) note that shrews, which are close rivals to hummingbirds in body size and metabolic rate, also have puzzling rest bouts that may be forced on them by digestive limitations.

More generally, young animals, because they are relatively small and susceptible to predators, resemble hummingbirds in being under pressure to acquire calories rapidly. While parents' ability to gather food (or the mother's ability to provide milk) may impose an ultimate limit on juvenile growth rates, this limit need not also be the proximate limit. In fact, infant rats that are fed with double the usual amount of milk grow no faster between four and ten days after birth than do normally fed infants (West et al. 1982). Thus, digestive physiology in some other cases may provide a proximate limiting factor for growth of young animals.

The examples of the preceding two paragraphs concern possible constraints that digestive physiology imposes in ecological time on the growth and behavior of individual animals. But digestive physiology may also constrain the evolutionary potential of species. If occupation of a new ecological niche would require genetic changes in digestive physiology, how easily can those genetic changes be acquired?

As one important example of this problem, consider the evolution of omnivory or herbivory. Ancestral vertebrates were carnivores, as most modern fishes, amphibia, and reptiles still are. For ancestral carnivores to cope with plant material, they had to acquire many new features of digestive physiology and anatomy. We have already mentioned one such feature, the ability to regulate apical glucose transporter activity according to dietary carbohydrate levels. As we noted, this regulatory apparatus is present in the omnivores and herbivores but absent in the strictly carnivorous families that we studied.

How many proteins and genes are involved in this apparatus? Do some carnivores already possess some of these proteins? Could some of the missing proteins be generated in a few steps by gene duplication and small changes in similar existing proteins, such as regulators of intestinal fructose transporters or of glucose transporters in organs other than intestine? If among carnivores some started out with more of the necessary proteins and were able to evolve the missing proteins in fewer steps, these species would have faced fewer inherent constraints to the evolution of herbivory.

At the molecular level, understanding of intestinal nutrient transporters and their associated digestive enzymes is growing rapidly. We hope that this knowledge will make it possible for physiologists to formulate questions about inherent constraints in experimentally testable terms and thereby to contribute to an important area of evolutionary biology.

Acknowledgments

It is a pleasure to acknowledge our debt to Roger Anderson, George Bartholomew, Peter Grant, Raymond Huey, Ernst Mayr, and two anonymous reviewers for suggestions on the manuscript. Charles R. Peterson graciously provided the garter snakes.

References cited

Arnold, S. J. 1981a. Behavioral variation in natural populations. I. Phenotypic, genetic, and environmental correlations between chemoreceptive responses to prey in the garter snake, *Thamnophis elegans*. *Evolution* 35: 489–509.

_____. 1981b. Behavioral variation in natural populations. II. Inheritance of a feeding response in crosses between geographic races of the garter snake, *Thamnophis elegans*. *Evolution* 35: 510–515.

Buddington, R. K. 1987. Does the natural diet influence the intestine's ability to regulate glucose absorption? *J. Comp. Physiol. B* 157: 677–688.

Buddington, R. K., J. W. Chen, and J. M. Diamond. 1987. Genetic and phenotypic adaptation of intestinal nutrient transport to diet in fish. *J. Physiol.* 393: 261–281.

Buddington, R. K., and J. M. Diamond. In press. Ontogenetic development of intestinal nutrient transporters. *Ann. Rev. Physiol.* 51.

Csaky, T. Z., and E. Fischer. 1981. Intestinal sugar transport in experimental diabetes. *Diabetes* 30: 568–574.

Diamond, J. M. 1986. Why do disused proteins become genetically lost or repressed? *Nature* 321: 565–566.

Diamond, J. M., and Buddington, R. K. 1987. Intestinal nutrient absorption in herbivores and carnivores. Pages 193–203 in P. Dejours, L. Bolis, C. R. Taylor, and E. R. Weibel, eds. *Comparative Physiology: Life in Water and on Land*. Springer-Verlag, New York.

Diamond, J. M., and W. H. Karasov. 1984. Effect of dietary carbohydrate on monosaccharide uptake by mouse small intestine in vitro. *J. Physiol.* 349: 419–440.

_____. 1987. Adaptive regulation of intestinal nutrient transporters. *Proc. Natl. Acad. Sci.* 84: 2242–2245.

Dykhuizen, D. 1978. Selection for tryptophan auxotrophs of *Escherichia coli* in glucose-limited chemostats as a test of the energy conservation hypothesis of evolution. *Evolution* 32: 125–150.

Ewald, P. F., and F. L. Carpenter. 1978. Territorial responses to energy manipulations in the Anna Hummingbird. *Oecologia* 31: 277–292.

Ferraris, R. P., and J. M. Diamond. 1986. Use of phlorizin binding to demonstrate induction of intestinal glucose transporters. *J. Membrane Biol.* 94: 77–82.

_____. In press. Specific regulation of intestinal nutrient transporters by their dietary substrates. *Ann. Rev. Physiol.* 51.

Ferraris, R. P., P. P. Lee, and J. M. Diamond. In press. Contributions of transporter number, transporter turnover number, and microvillus area to species differences in intestinal transport. *Am. J. Physiol.*

Ginsburg, J. M., and F. W. Heggeness. 1968. Adaptation in monosaccharide absorption in infant and adult rats. *J. Nutr.* 96: 494–498.

Hanski, I. 1984. Food consumption, assimilation and metabolic rate in six species of shrew (Sorex and Neomys). *Ann. Zool. Fennici* 21: 157–165.

Hediger, M. A., M. J. Coady, T. S. Ikeda, and E. M. Wright. 1987. Expression cloning and cDNA sequencing of the Na+/glucose cotransporter. *Nature* 330: 379–381.

Hixon, M. A., F. L. Carpenter, and D. C. Paton. 1983. Territory area, flower density, and time budgeting in hummingbirds: an experimental and theoretical analysis. *Am. Nat.* 122: 366–391.

Johnson, L. R. 1987. *Physiology of the Gastrointestinal Tract*. Raven Press, New York.

Karasov, W. H. 1987. Nutrient requirements and the design and function of guts in fish, reptiles, and mammals. Pages 181–191 in P. Dejours, L. Bolis, C. R. Taylor, and E. R. Weibel, eds. *Comparative Physiology: Life in Water and on Land*. Springer-Verlag, New York.

_____. 1988. Nutrient transport across vertebrate intestine. Pages 131–172 in R. Gilles, ed. *Advances in Comparative and Environmental Physiology*. Springer-Verlag, Berlin, FRG.

Karasov, W. H., and E. S. Debnam. 1987. Rapid adaptation of intestinal glucose transport: a brush-border or basolateral phenomenon? *Am. J. Physiol.* 253: G54–G61.

Karasov, W. H., and J. M. Diamond. 1983a. Adaptive regulation of sugar and amino acid transport by vertebrate intestine. *Am. J. Physiol.* 245: G443–G462.

_____. 1983b. A simple method for measuring solute uptake by intestine in vitro. *J. Comp. Physiol. B* 152: 105–116.

_____. 1985. Digestive adaptations for fueling the cost of endothermy. *Science* 228: 202–204.

Karasov, W. H., D. Phan, J. M. Diamond, and F. L. Carpenter. 1986. Food passage and intestinal nutrient absorption in hummingbirds. *Auk* 103: 453–464.

Karasov, W. H., R. S. Pond III, D. H. Solberg, and J. M. Diamond. 1983. Regulation of proline and glucose transport in mouse intestine by dietary substrate levels. *Proc. Natl. Acad. Sci.* 80: 7674–7677.

Karasov, W. H., D. H. Solberg, and J. M. Diamond. 1985a. What transport adaptations enable mammals to absorb sugars and amino acids faster than reptiles? *Am. J. Physiol.* 249: G271–G283.

Karasov, W. H., D. H. Solberg, S. D. Chang, M. Hughes, E. D. Stein, and J. M. Diamond. 1985b. Is intestinal transport of sugar and amino acids subject to critical-period programming? *Am. J. Physiol.* 249: G770–G785.

Krebs, J. R., and N. B. Davies. 1984. *Behavioural Ecology*. Blackwell Scientific Publ., Oxford, UK.

Krebs, J. R., and P. H. Harvey. 1986. Busy doing nothing—efficiently. *Nature* 320: 18–19.

Kuno, Y. 1956. *Human Perspiration*. Charles C. Thomas, Springfield, IL.

Maenz, D. D., and C. I. Cheeseman. 1986. Effect of hyperglycemia on D-glucose transport across the brush-border and basolateral membrane of rat small intestine. *Biochim. Biophys. Acta* 860: 277–285.

Ridley, M. 1983. *The Explanation of Organic Diversity*. Clarendon Press, Oxford, UK.

Rubenstein, D. I., and R. W. Wrangham. 1986. *Ecological Aspects of Social Evolution*. Princeton University Press, Princeton, NJ.

Scharrer, E. 1976. Developmental changes of sugar transport in the ovine small intestine. *Pfluegers Arch. Eur. J. Physiol.* 366: 147–151.

West, D. B., J. Diaz, and S. C. Woods. 1982. Infant gastronomy and chronic formula infusion as a technique to overfeed and accelerate weight gain of neonatal rats. *J. Nutr.* 112: 1339–1343.