Rapid upregulation of snake intestine in response to feeding: a new model of intestinal adaptation

STEPHEN M. SECOR, ERIC D. STEIN, AND JARED DIAMOND

Department of Physiology, University of California at Los Angeles Medical School, Los Angeles, California 90024-1751

Secor, Stephen M., Eric D. Stein, and Jared Diamond. Rapid upregulation of snake intestine in response to feeding: a new model of intestinal adaptation. Am. J. Physiol. 266 (Gastrointest. Liver Physiol. 29): G695–G705, 1994.—Mammalian guts exhibit numerous adaptive responses to feeding. However, response magnitudes are often inconveniently modest for experimental analysis, because mammals feed often and their intestines are rarely empty. We anticipated larger responses in sit-and-wait foraging snakes, because they consume huge meals at long intervals. Hence, we studied metabolic rates, brush-border nutrient transport, and intestinal morphometrics in the rattlesnake, Crotalus cerastes, as a function of time since feeding. O2 consumption by the whole snake, a reflection of the cost of digestion and of rebuilding the starved gut, peaked after 2 days at eight times fasting values. Activities of brush-border glucose, leucine, and proline transporters peaked after 1–3 days at 5–22 times fasting values. Ratios of amino acid to glucose uptake rates peaked at 104, reflecting snakes’ extreme adaptation to carnivory (a high-protein low-carbohydrate diet). Intestinal mass increased more than twofold within 1 day, primarily because of mucosal growth. After defecation, the intestine atrophied, brush-border transporters were downregulated, and O2 consumption returned to basal. These rapid and large responses reduce costs of gut maintenance during long bouts of quiescence between meals. Hence sit-and-wait foraging snakes may furnish advantageous model species for studying gut regulation and adaptation.

brush border; sugar transport; amino acid transport; specific dynamic action, intestinal hypertrophy

DIGESTIVE MECHANISMS of the gut adapt on various time scales to fluctuations in dietary nutrient inputs. Familiar examples of these food-triggered regulatory responses include the modulation of gastric, pancreatic, and intestinal enzyme and electrolyte secretion within a few minutes by nerves and hormones (42); substrate-dependent induction of enzyme and transporter synthesis within a day (11); slower atrophy of the gut with starvation, and hypertrophy of the gut with the hyperphagia associated with lactation and cold stress (17).

Most mammal and bird species, however, consume relatively small meals at frequent intervals. Because the pyloric sphincter meters food release into the duodenum, intestinal nutrient concentrations fluctuate only modestly over 24 h, even in rodents whose food consumption is mainly restricted to night hours (12). Thus some phase of digestion is usually underway, and ranges of digestive activities from minimal to peak values are modest. For example, activities of intestinal brush-border sugar and amino acid transporters in most mammals vary only up to twofold with variation in dietary carbohydrate and protein levels (11). Increases in metabolic rate during digestion, termed specific dynamic action, are only by 25–50% (4). The molecular mechanisms underlying such regulatory responses would be easier to study if the responses were larger.

At the opposite extreme from most mammals in meal frequency and meal size are snakes that sit and wait in ambush for their prey (25). Field studies of individual radiotelemetered viperid snakes (rattlesnakes and vipers) and boid snakes (boas, pythons, and anacondas) reveal that they capture a prey animal once every month or two while active (30, 33) and that pregnant females may omit feeding for 4–18 mo before giving birth (23, 40). Captive snakes have been observed to survive fasts of 14–30 mo, far longer than the fasts required to kill any mammal or bird. Ambush-hunting snakes compensate for low meal frequency with large meal size, often over 50% and even up to 160% of empty body weight (13). Digestion is much slower than in most mammals, transit times from mouth to anus being measured in weeks for viperid and boid snakes (21, 27) as opposed to hours for small mammals. In short, the guts of such snakes are adapted to being inactive for a long time, then consuming an enormous meal and spending a long time digesting it.

The active intestine has high maintenance costs, because enterocytes have one of the most rapid turnover rates of any tissue (15). One might therefore expect fasting snakes to save energy by downregulating gut activities and by upregulating them rapidly upon capture of prey. If so, ranges of digestive activities from minimal to peak values might be much greater than in mammals. If the molecular mechanisms in snake intestines were nevertheless similar to those in mammalian intestines and differed mainly in much greater regulatory span, snake intestines might furnish useful models to gastrointestinal physiologists for studying adaptive regulation, analogous to the role of the squid axon in neurophysiological research.

We have tested this possibility by studying feeding responses of a species of rattlesnake, the sidewinder (Crotalus cerastes). We chose this species because one of us had already measured its natural feeding intervals in the wild (30). As expected, we found that it responds to feeding with increases in metabolic rate and in brush-border nutrient transporter activities far greater than those observed in most mammals. Yet its transporters seem, in other respects examined, similar to those of other vertebrates. Thus snakes may indeed prove to be a fruitful model species in gastrointestinal research. Poisonous rattlesnakes themselves are unlikely to become the preferred model species, but we have subsequently found equally large or even larger regulatory responses
in nonpoisonous snake species (Secor and Diamond, unpublished data).

**MATERIALS AND METHODS**

We describe in turn animals and their maintenance; our methods for measuring food passage rate, metabolic response to feeding, nutrient uptake, and morphometric parameters; solutions and chemicals; and statistical methods.

**Animals and Their Maintenance**

Sidewinders inhabit sandy areas of the deserts of southwestern North America (21). Waiting stationary in ambush, they strike their prey (mainly lizards and small rodents) and kill by injecting venom. Radiotelemetered sidewinders were observed to consume on the average one rodent every 1–2 mo during their activity season (30).

We captured 35 sidewinders (18 males, 17 females) from around the Kelso Dunes in San Bernardino County, California. Ranges of measurements were as follows: mass, 33–199 g [97 ± 7 (SE) g]; total length, 40–67 cm [53 ± 1 (SE) cm]; snout-to-vent length, 37–63 cm [49 ± 1 (SE) cm]. These measurements, the month of capture (September), and observations of spermatogenesis in testes of males imply that all snakes were at least 1 yr old, and most were sexually mature.

Snakes were housed in individual plastic boxes (30 x 15 x 7 cm) with water available ad libitum, within an environmental chamber at 30°C (the mean body temperature of wild sidewinders that have fed recently; Secor, unpublished data). Because we found that sidewinders complete digestion within 2 wk of ingestion, we withheld food from snakes for 3 wk after capture to ensure that they were all in a postdigestive state. We then offered snakes one or two live mice (Mus musculus) with a combined weight of 26.4 ± 0.4% of the snake’s body mass, a typical value for wild sidewinders (5–82%; Secor, unpublished data). Snakes were then studied at various times after feeding, as we now describe.

**Food Passage Rate**

We measured rates of digestion and food passage by two methods. 1) X-ray photographs were taken of four snakes at 1, 3, 6, 8, 10, and 14 days after feeding. At each time we took an X-ray of the midsection including the stomach and intestine and estimated the percentage of the mouse’s skeleton still within the snake’s stomach. 2) When we killed each snake, we weighed both the stomach and intestinal contents and expressed them as a percentage of the original meal mass.

**Metabolic Response to Feeding**

We used closed-system respirometry (see Ref. 39 for details) to measure O2 consumption rates of 12 snakes daily for 4 days before and 12–25 days after feeding. Snakes were placed individually within opaque chambers (1,500 or 3,000 ml) inside a temperature-controlled environmental cabinet at 30°C. Air samples were drawn at the beginning and end of a period of 1–2 h, during which the chambers were sealed. The fractional O2 concentrations of the air samples were measured with an Applied Electrochemistry S-3A O2 analyzer, and O2 consumption rates were calculated from Eq. 9 of Vleck (39).

**Nutrient Uptake Measurements**

**Methods.** A snake was killed by transecting the spinal cord immediately posterior to the skull. The intestine was rapidly removed, washed out with ice-cold Ringer solution (see Solutions and Chemicals for composition), everted, and cut into sleeves 1 cm long, which were kept in ice-cold oxygenated Ringer solution until ready to be mounted and incubated.

We measured nutrient uptake across the brush-border membrane of the intestinal mucosa (not across the whole epithelium), as described in detail by Karasov and Diamond (18). Briefly, an everted 1-cm sleeve of intestine (mucosal surface facing outward) mounted on a grooved glass rod was preincubated for 5 min in Ringer solution at 30°C, then incubated for 2 min at 30°C in Ringer solution containing a radiolabeled nutrient (50 mM L-[3H]proline, 50 mM L-[3H]leucine, trace concentrations of L-[14C]glucose, or 20 mM D-[14C]glucose), plus an appropriate corresponding adherent fluid marker at trace concentrations ([14C]polyethylene glycol (PEG) with the 3 first-named solutes, or L-[14C]glucose with D-[14C]glucose). In each case the nutrient was the solute whose uptake was being measured, while the marker solute served to correct for the quantity of the nutrient in the adherent fluid. In addition, L-[14C]glucose corrects for passive D-[14C]glucose uptake. The choice of incubation times, concentrations, and temperature was based on validation experiments described in the APPENDIX. These four pairs of solutes yielded, respectively, the total uptake (carrier-mediated plus passive diffusional uptake) of L-proline and L-leucine, passive uptake of D or L-glucose, and carrier-mediated uptake of D-glucose. Na+-independent uptake of L-proline or L-leucine was determined by preincubating and incubating sleeves in Na+-free solutions (NaCl replaced isosmotically with choline chloride). Na+-independent uptake of these amino acids was calculated as total uptake measured in Na+-containing solutions minus Na+-independent uptake measured in choline-containing solutions.

**Intestinal regions.** The small and large intestines were easily distinguished in sidewinders; the large intestine had a wider diameter, smoother texture, and straight course, unlike the bends of the thinner small intestine. At ~50–70% of the distance along the length of the small intestine from the pyloric sphincter, it changed abruptly in appearance; it decreased in diameter, the mucosal layer became thinner, and the color changed from fluffy white to pale pink. Hence, we divided the small intestine into three regions: the morphologically distinctive distal region and proximal and middle regions, defined as equal-length halves of the proximal 50–70%.

**Expression of uptakes.** We normalized measured nutrient uptake rates to the wet mass of the everted sleeve and expressed them as nanomoles per minute per milligram. To calculate the total uptake capacity (μmol/min) of the whole length of the small intestine, we calculated the uptake capacity for each small intestinal region as the average uptake rate (nmol min⁻¹ mg⁻¹) multiplied by that region’s wet mass, then summed those products over the intestine’s regions (9).

**Schedule of measurements.** To study the intestinal response to feeding, we measured nutrient uptake and morphometrics in 21 snakes: 3 each killed at 0.5, 1, 3, 6, 14, 30, and 40 days after feeding. The mean time of defecation was 12.6 ± 0.7 days (n = 9) after feeding so that measurements through 6 days were of digesting animals, measurements at 14 days were immediately postdigestive, and those at 30 and 40 days were of fasted animals. Snakes were grouped so that there was no significant difference by analysis of variance (ANOVA) in body mass (F6,14 = 1.55, P = 0.23) or snout-vent length (F6,14 = 1.28, P = 0.33) among the sets of three individuals used for the seven sampling periods.

**Morphometrics.** To quantify feeding responses of intestinal morphology at the gross and light-microscopic levels, we made four sets of...
FEEDING RESPONSES OF SNAKE INTESTINE

measurements on the 21 snakes killed at the 7 sampling periods. 1) We measured the length and wet mass of the entire small intestine immediately after excising it, then the wet masses of the 1-cm sleeves used in uptake experiments. 2) We determined the proportion of intestinal mass arising from scrapable mucosa, by using a glass slide to scrape the mucosa from the serosa of 1-cm sleeves from each small intestinal region (9). The scrapable mucosa and residual serosa were dried (60°C) and weighed. Mucosal and serosal wet masses were then calculated on the assumption that both layers had the same dry-to-wet mass ratio. 3) We measured the ratio of intestinal mucosal to serosal circumference in light-microscopic sections, as a reflection of elaborations of the luminal (mucosal) surface. 4) In light-microscopic slides we measured heights and diameters of five enterocytes from each intestinal region of each snake.

In other vertebrate species, hyperphagia and fasting are, respectively, associated with growth and atrophy of other organs in addition to the intestine (38). Hence, we measured wet masses of the following organs: empty stomach, empty large intestine, full gallbladder, heart, liver, kidneys, lung (most snakes have a single lung), and abdominal fat bodies.

Solutions and Chemicals

We used a Ringer solution with an osmolarity resembling rattlesnake plasma (Ref. 24; 300 mosM) and an electrolyte composition as follows (in mM): 128 NaCl, 20 NaHCO3, 4.7 KCl, 2.5 CaCl2, 1.2 KH2PO4, and 1.2 MgSO4, pH 7.4 when aerated with 95% O2-5% CO2. When nutrients were added, NaCl concentration was reduced so as to maintain constant osmolarity. Reagents were of the purest quality available (Sigma Chemical). Isotopes were from New England Nuclear, Amersham, and ICN Biochemicals.

Statistics

For most variables we tested for differences among sampling periods by a one-way ANOVA. However, variation in body mass proved to have significant effects on total proline and leucine uptake capacity and on intestinal, mucosal, and organ masses, so we removed its effect by analysis of covariance (ANCOVA) with body mass as the covariate. In all such ANCOVA analyses, we found no significant interaction between body mass and day postfeeding. We used a repeated-measures-design ANOVA to test for differences among days pre- and postfeeding in metabolic response and among intestinal positions (43). We present our results as means ± SE or as adjusted means (so-called least-squares means provided by ANCOVA) ± SE. Results of statistical tests are stated in the text in terms of their F and P values. We used a Tukey’s multiple-range test as a means separation test to identify significant differences between individual pairs of values within data sets (43). The level of significance was taken as P < 0.05.

RESULTS

Food Passage Rate

Mice were swallowed head first. At 1 day postfeeding, X-ray photographs and stomach content weights revealed that the whole mouse was still within the snake’s stomach (Fig. 1). At 3 days, the mouse’s posterior half was still intact in the stomach, but the skull had been digested, and about one-eighth of the mouse by weight was now in the snake’s intestine. At 6 days, the mouse’s forelimbs and thoracic cavity had been digested, and the snake’s intestinal content mass peaked at about one-fifth of the mouse’s weight. At 10 days, the sole skeletal elements still occasionally present in the snake’s stomach were hindlimbs and caudal vertebrae. At 14 days, 1 or 2 days after the normal time of defecation, the stomach contained no bones and only small mats of hair, and only 15% of the ingested mouse mass remained within the snake’s stomach and intestines.

Metabolic Response to Feeding

O2 consumption rates before feeding were 0.030 ± 0.007 ml O2 g-1 h-1 (Fig. 2). Rates rose abruptly after feeding to a value 6.6-fold higher at day 1 and to a peak value 7.8-fold higher (0.235 ± 0.007 ml O2 g-1 h-1) at day 2. Rates began to decline significantly below this
Anterior uptakes of all three solutes were much higher at 1 day than 0.5 days postfeeding (Tukey's multiple-range test, $P < 0.05$). For L-leucine and D-glucose, anterior uptakes peaked at 1 day at values 7.8 and 5.4 times the 0.5-day values (Fig. 3). Anterior uptakes of these two solutes at day 3 were significantly below these peak values. For L-proline, anterior uptake peaked at day 3 at 6.6 times the 0.5-day value. By day 3 (D-glucose) or day 14 (L-proline and L-leucine), anterior uptakes were back down to 0.5-day values and did not vary significantly thereafter through day 40.

In the distal small intestine, uptake did not vary significantly ($P > 0.1$) with days postfeeding for L-proline or L-leucine. Distal glucose uptake exhibited the same pattern as anterior glucose uptake, peaking at day 3 at 14.6 times the 0.5-day value.

Figure 4 depicts calculated ratios of total amino acid uptake to glucose uptake by anterior intestine, for comparison with published values for other vertebrate species. The ratio greatly exceeded 1.0 for both amino acids at all sampling times. It peaked at day 6, at a value of $104 \pm 19$ for L-proline/D-glucose and $70 \pm 15$ for L-leucine/D-glucose.

Na$^+$ dependence of amino acid uptake. Both the Na$^+$-dependent and Na$^+$-independent components of L-proline and L-leucine uptake in the anterior small intestine varied significantly with day postfeeding ($P < 0.0001$) and peaked at either day 1 or day 3 (Fig. 5). The ratios of the peak values to the 0.5-day values were 16.2, 13.4, 5.1, and 3.7 for Na$^+$-dependent proline, Na$^+$-dependent leucine, Na$^+$-independent proline, and Na$^+$-independent leucine uptakes, respectively; that is, the rise was steeper for the Na$^+$-dependent components, which accounted for 41 ± 3 and 61 ± 5% of total uptake on day 1 for proline and leucine, respectively. On other days, the Na$^+$-dependent component declined to 20 ± 3 and 19 ± 8% for proline and leucine, respectively.

Uptake capacities. If one integrates uptake rates (nmol·mg$^{-1}$·min$^{-1}$, as illustrated in Fig. 3) over the...
whole length of the small intestine as described in METHODS, one obtains the total uptake capacity of the small intestine (Fig. 6). Capacity rose steeply after feeding and peaked at day 1 (D-glucose, L-leucine) or days 1–6 (L-proline). It quickly returned to the 0.5-day value by day 3 for D-glucose, but not until after day 6 for the two amino acids. The ratio of the peak value to the 0.5-day value was 6.9 for D-glucose, 5.7 for L-leucine, and 3.9 for L-proline. These increases in uptake capacity are due primarily to the increases in uptake rates illustrated in Fig. 3, secondarily because of an increase in intestinal mass (Fig. 7).

**Intestinal histology.** The ratio of mucosal to serosal circumference seen in microscopic slides is a measure of elaborations and foldings of the mucosal surface at the light microscopic level (Fig. 9). Ratios differed with.

**Morphometrics**

**Intestinal mass.** Small intestinal length did not vary with days postfeeding, but small intestinal mass did ($F_{0.13} = 4.35, P < 0.05$). The value at 0.5 days (6.2 ± 0.7 g) was already higher ($P < 0.05$) than the day 40 value (2.6 ± 0.7 g), remained high at day 1 (6.3 ± 0.7 g), then declined slowly. The only significant differences were between the day 40 value and the 0.5- or 1-day values. Changes in small intestinal mass were due entirely to changes in anterior small intestine, whose mass (mg/cm) peaked at day 1, more than twice the day 40 value (Fig. 7). The distal small intestine experienced no change in mass.

These changes in anterior intestinal mass were in turn due largely to changes in the intestinal mucosa rather than in the serosa. At day 1, the anterior mucosal mass peaked at a value three times the day 40 value and did not return to a value statistically inseparable from the day 40 value until day 14 (Fig. 8). Distal mucosal mass was much smaller and did not vary with day of sampling (Fig. 8).

**Fig. 5.** Na⁺-independent and Na⁺-dependent components of proline (A) and leucine (B) uptake in anterior intestine, as a function of days after feeding. Note that both components for both amino acids rise to peaks at day 1 or 3, and that Na⁺-independent uptake predominates except for leucine at days 1 and 6.

**Fig. 6.** Total uptake capacities of entire length of small intestine for proline, leucine, and glucose as a function of days after feeding. Note that proline and leucine values greatly exceed glucose values and that values for all 3 nutrients rise steeply to a peak after feeding (mainly because of increases in uptake rates; Fig. 3), secondarily because of an increase in intestinal mass (Fig. 7).

**Fig. 7.** Average wet masses (mg/cm) of anterior and distal small intestine, as a function of days after feeding. Note that anterior mass peaks at day 1 and that distal mass is less and does not change.
FEEDING RESPONSES OF SNAKE INTESTINE

Fig. 8. Mucosal and serosal wet mass (mg/cm) of anterior and distal small intestine, as a function of days after feeding. Note that mucosa is relatively and absolutely much heavier anteriorly than distally and that anterior mucosal mass peaks at day 1, while anterior serosal mass and distal masses do not change.

Fig. 9. Ratio of mucosal-to-serosal circumference of each small intestinal region, as a function of days after feeding. This ratio expresses membrane elaborations and foldings of mucosal surface. Note that ratios are higher in proximal and middle than distal intestine, that they rise and then decline again after feeding, and that they peak earlier in proximal and middle intestine (day 6) than in distal intestine (day 14).

Fig. 10. Enterocyte size and number, averaged over all 3 regions of small intestine, as a function of days after feeding (n = 5 cells for each region for each of 3 snakes). From enterocyte heights and diameters (A and B), cell volume (C) was calculated from formula for a cylinder, as \( \pi d^2 h \), where \( d \) is diameter and \( h \) is height. Cell number (D, cells/cm length of intestine) was then calculated as mass of whole mucosa (from Fig. 8) divided by mass of an individual cell (from C, assuming 1 µl of cells ~1 mg). We did not measure mucosal mass at 0.5 days; hence cell number was not calculated then.

Enterocyte heights and diameters (Fig. 10, A and B) varied with sampling day (\( P < 0.05 \)) and intestinal region (\( P < 0.001 \)). Averaged over all days, the tallest cells were those of the distal intestine (55 ± 3 µm, compared with 50 ± 2 µm for middle and 42 ± 1 µm for proximal intestine), while the greatest cell diameters were in the middle region (5.7 ± 0.4 µm, compared with 5.2 ± 0.3 µm for distal and 4.7 ± 0.3 µm for proximal intestine). Averaged over all three small intestinal regions, cell height and diameter both peaked at day 6, at values 1.3 and 2.0, respectively, times the day 40 values (Fig. 10). Cell volume (Fig. 10C) peaked at day 6 at a value 5.7 times the day 40 value. Cell number actually declined to a minimum value at day 6 (Fig. 10D), because the increase in mucosal mass (Fig. 8) was by a smaller factor than the increase in cell volume (Fig. 10C); that is, the increase in mucosal mass was due to cell hypertrophy, not to mucosal hyperplasia.

Organ masses. Apart from these changes in small intestinal mass among sampling periods, changes in masses of other organs (stomach, large intestine, gallbladder, liver, fat bodies, heart, kidneys, and lung) did not achieve statistical significance. Suggestive trends were a decline in liver mass over the 40 days of sampling period (\( P < 0.001 \)).
and a nearly twofold decline in mass of fat bodies over the first 3 days followed by recovery after day 14. Larger sample sizes would be required to assess the reality of these possible changes.

**DISCUSSION**

We shall discuss in turn food passage rate; the metabolic response to feeding; responses of intestinal morphology, transporter activities, and transporter capacities to feeding, comparisons of sidewinders with other carnivorous vertebrates with respect to intestinal transport; the adaptive significance of our findings; and unresolved problems.

**Food Passage Rates**

The time from ingestion to defecation for sidewinders averaged 12.6 days. This is slow even by the standards of other similar-sized snakes, which are already much slower than endotherms. For example, at 25–31°C, all bones of the prey are digested within 2.5 days in garter snakes (*Thamnophis elegans*; Ref. 36) and grass snakes (*Natrix natrix*; Ref. 32), as opposed to 10 days or more for sidewinders (this study). Defecation occurred at 3–5 days for the former two species, only 30–40% of the time required for sidewinders. The slowness of digestion in sidewinders may be related to their low resting metabolic rate (low even by snake standards) and to the long periods of inactivity between their infrequent feeding bouts. Sidewinder intestine atrophies during these periods of inactivity, and the need to upregulate and rebuild the intestine upon feeding delays digestion.

**Metabolic Response to Feeding**

The increase in metabolic rate associated with digestion is termed specific dynamic action. Typical values for the increase are by 25–50% in mammals (4) and by 82–865% in the 10 species of reptiles studied to date (2, 41). The value for sidewinders, 680%, is the largest reported for any species.

One reason for this large percentage increase is the low resting metabolic rate of fasting sidewinders, only 35% of the value predicted from the allometric equation for resting fasted snakes of other species (3). A second reason is the high metabolic rate of fed sidewinders: 0.23 ml O₂·g⁻¹·h⁻¹ for stationary sidewinders 2 days after feeding, equivalent to the value for fasted sidewinders traveling at their normal speed of 0.1 km/h (29).

The postfeeding metabolic spurt in sidewinders presumably serves to fuel the immediate costs of digestion (including gastric acid secretion), plus the costs of preparing the gut for digestion (including intestinal growth and transporter upregulation). The "extra" O₂ consumption (beyond preingestion levels), summed over the time from day 0 (when the snake swallows the mouse) to day 9 (when O₂ consumption no longer differs significantly from preingestion levels), is ~25 ml O₂/g. If we assume that sidewinders during this period are metabolizing a mixture of fat and protein (respiratory quotient = 0.75) and that 1 ml CO₂ produced corresponds to an energy yield of 25.7 J (26), then the extra energy expended during digestion for a 140-g sidewinder is ~66 kJ. This represents 26% of the assimilated energy from a typical prey item of a 35-g mouse, assuming 8.95 kJ/g energy yield from a mouse (34) and 80% assimilation efficiency for a sidewinder (30). Figure 2 makes clear that much of this energy cost of digestion is incurred within the first few days, while the mouse is still in the snake’s stomach and before intestinal absorption of energy from the mouse has begun. Hence, this initial cost of digestion (for gastric acid secretion and rebuilding the intestine) must be fueled by mobilization of the snake’s own energy reserves, especially its fat bodies and muscle protein.

**Intestinal Responses to Feeding**

Upon feeding, sidewinders undergo abrupt increases, and subsequent slower declines, of intestinal mass and transporter activities per unit mass, hence of total intestinal uptake capacity.

The increase in intestinal mass is visible to the eye, as an increase in thickness of the small intestine’s longitudinal folds, which serve to increase mucosal surface area and are thus functionally equivalent to the villi of mammalian small intestine. We obtained four measurements of this intestinal growth: intestinal mass (Fig. 7), mucosal mass (Fig. 8), mucosal circumference (Fig. 9), and enterocyte height and diameter and hence volume (Fig. 10). This intestinal hypertrophy in sidewinders is triggered by ingestion of a single large bolus of food. Analogously, intestinal growth in mammals accompanies increased sustained levels of food intake during pregnancy (8), lactation (14), and cold exposure (38).

Activities of the glucose, proline, and leucine transporters rose rapidly within 24 h of feeding to peak values five to eight times the fasted values (Fig. 3). While both the Na⁺-dependent and Na⁺-independent components of proline and leucine transport were upregulated, the larger increase was in the Na⁺-dependent components, by 12 to 22 fold (Fig. 5). For comparison, the glucose and amino acid transporters of rodent intestine are regulated over only a twofold range of activities (11). The regulatory spans of the glucose and amino acid transporters in sidewinder intestine are among the largest known to us for intestinal transport of any macronutrient and are surpassed only by upregulation of the glucose transporter in Burmese pythons (*Python molurus*) (S. M. Secor and J. M. Diamond, unpublished data) and in sheep (31). The increases in sidewinder intestinal uptake capacity for glucose and amino acids (Fig. 6) are due primarily to these increases in transporter activities and secondarily to the increase in intestinal mass discussed in the preceding paragraph.

The surges in proline and leucine transport do not return to basal levels until day 14 after feeding. Thus upregulation of these amino acid transporters coincides with the duration of intestinal digestion and undoubtedly serves the function of enhanced absorption of amino acids released from the prey’s protein. However, upregulation of the glucose transporter must serve some function other than enhanced absorption of dietary substrate, since rodent carcasses contain little carbohy-
drate, and since glucose transport returns to basal levels within 3 days, at a time when digested matter is beginning to enter the small intestine. We hypothesize that the rapid surge in metabolic rate upon feeding arises from mobilization of the snake’s energy resources to rebuild the intestine, that this mobilization involves raised levels of plasma glucose, and that the function of glucose transporter upregulation is to reabsorb glucose diffusing from the plasma down its concentration gradient into the intestinal lumen. This hypothesis is supported by the observation that glucose transporter upregulation takes place not only in the anterior small intestine but also in the morphologically distinctive distal small intestine, which does not participate in upregulation of intestinal mass and amino acid transport.

Comparisons With Intestines of Other Carnivorous Species

Intestinal mass. Endotherms sustain time-averaged metabolic rates ~10 times higher than similar-sized ectotherms. To fuel these higher metabolic rates, endotherms have correspondingly higher food intakes. The principal intestinal adaptation permitting endotherms to process food more quickly is their relatively much greater intestinal mass (20). As Table 1 illustrates, small intestinal mass comprises ~1% of body mass for three carnivorous ectotherm species but ~5% for three carnivorous endotherm species, all of which feed at frequent intervals on small prey items. Interestingly, the value for sidewinders (5.3% for digesting snakes, 3.7% for fasting ones) falls within the range for endotherms.

Effects of small intestinal position. Glucose and amino acid uptakes are markedly lower in sidewinder distal small intestine than in anterior intestine. Similar gradients of uptake are observed in all carnivores and many herbivores studied to date (e.g., Refs. 6, 10, 20).

Glucose transporter. The intestinal glucose transporter of sidewinders (see APPENDIX) resembles that of other vertebrate species in its stereospecificity for D-glucose, its apparent Michaelis constant value of 1.4 mM (see Refs. 5, 16, and 37 for values for other vertebrates), and its molecular structure (as deduced from the detection of its mRNA by probes of the glucose transporter of rabbit intestine; L. Lescale-Matys, unpublished data). As true for other carnivores as well, the maximum velocity value for the sidewinder glucose transporter (0.15 nmol·mg⁻¹·min⁻¹ at 3 days postfeeding) is an order of magnitude lower than that for herbivores (typically 2–3 nmol·mg⁻¹·min⁻¹), correlated with the much lower carbohydrate content of carnivores’ diets (19).

Intestinal transporter activities and mass-normalized uptake capacities (defined as capacity/body mass⁰.⁷⁵; Ref. 28) are generally much lower in ectotherms than in endotherms, correlated with the lower food intakes of ectotherms (6, 7, 16, 19, 37). Glucose transport rates in sidewinders at most times in the feeding cycle are comparable to the low values of fed individuals of other ectothermic carnivore species (Table 1). However, peak rates in sidewinders 1 day after feeding are much higher and approach or reach those of endothermic carnivores (Table 1). This conclusion holds, whether one bases the comparison on uptake rates per milligram intestine or on mass-normalized uptake capacities.

Passive glucose permeability. The passive glucose permeability coefficient (Kₚ) for sidewinder intestine is 0.01 μmol·min⁻¹·mg⁻¹, similar to values for other vertebrate species (10, 20, 22). Passive permeation accounts for 75% of glucose uptake at 20 mM in sidewinders at all times except day 1, when the passive component declines to 25% because of the surge in active glucose uptake. If plasma glucose rises at day 1, and if this surge in active glucose uptake did not occur, then the high value of passive permeability relative to active glucose uptake except at day 1 would mean large losses of plasma glucose into the intestinal lumen; that is, our

Table 1. Comparison of carnivores

<table>
<thead>
<tr>
<th></th>
<th>EcotHERMs</th>
<th>EndothereMs</th>
<th>Sidewinder (ND/D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trout</td>
<td>Leopard</td>
<td>Bullfrog</td>
</tr>
<tr>
<td>Body mass, g</td>
<td>300</td>
<td>36</td>
<td>147</td>
</tr>
<tr>
<td>Intestinal mass, g</td>
<td>2.2</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Intestinal mass to body mass ratio</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Uptake rate, nmol·min⁻¹·mg⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>0.03</td>
<td>0.04</td>
<td>0.25</td>
</tr>
<tr>
<td>L-Proline</td>
<td>0.39</td>
<td>0.90</td>
<td>1.25</td>
</tr>
<tr>
<td>L-Leucine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normalized uptake capacity, μmol·min⁻¹·body mass⁰.⁷⁵</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>L-Proline</td>
<td>0.04</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>L-Leucine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparisons of sidewinder intestine with intestines of 3 other carnivorous ecotthers and 3 carnivorous endotherms are shown. ND, nondigesting sidewinders (average values for 1, 3, and 6 days postfeeding). Data for trout, leopard frog, mink, cat, bullfrog, and house wren are from Refs. 7, 6, 6, 6, 37, and 10, respectively, and from the present paper for sidewinders. In several respects (especially intestinal mass to body mass ratio and amino acid uptake rates and capacities), intestine of sidewinder (especially digesting sidewinders, D) is more similar to endotherm intestines than to intestines of other ectotherms.
measured value of \( K_a \) reinforces our interpretation that the surge in active glucose uptake at day 1 is adaptive.

**Amino acid transporters.** Proline and leucine transport rates in fed sidewinders exceed those even in endothermic carnivores and far exceed those in ectothermic carnivores (Table 1). Even fasted sidewinders have rates approaching or reaching values for endothermic carnivores (Table 1). These conclusions apply, whether one bases the comparison on uptake rates per milligram intestine or on mass-normalized uptake capacities.

Transport of proline and leucine by sidewinders involves both Na\(^+\)-dependent and Na\(^+\)-independent components, as in intestines of other vertebrates. Available data indicate that the Na\(^+\)-dependent component for proline predominates in almost all herbivorous and omnivorous species, while the trend is for the Na\(^+\)-independent component to predominate in carnivores (10, 20, 22, 37). Sidewinders fit this pattern, since the Na\(^+\)-independent component accounts for 76 and 75% of proline and leucine uptake, respectively, at 50 mM. The Na\(^+\)-dependent component appears saturable (see Appendix) and presumably wholly carrier mediated. While part of the Na\(^+\)-independent component could be by passive diffusion, it too is saturable and thus is at least in part carrier mediated, as well established for Na\(^+\)-independent amino acid uptake in rabbit intestine (35).

**Ratios of amino acid to glucose transport.** Carnivores generally have a diet high in protein and low in carbohydrate; hence their intestines generally have higher activities of amino acid transporters than of the glucose transporter. The reverse facts generally apply to herbivores. Ratios of proline to glucose uptake capacity (P/G ratio) are 1–10 for most carnivores and <1 for herbivores and omnivores (6, 10, 19). Sidewinders represent an extreme even among carnivores, with a P/G ratio that peaks at 104 in digesting individuals and declines to 38 in nondigesting individuals, values matched by only one other carnivorous species studied to date (the American toad; Ref. 19).

**Adaptive Significance**

The distinctive features of sidewinders’ feeding habits, shared with other species of sit-and-wait foraging snakes, are their relatively huge meals consumed and their long intervals between those meals. The intervals vary predictably with season, vary with unpredictable variations in prey density, and exceed 1 yr under some conditions. As a result, the intestine sees no food for most of the time, then is presented with a huge bolus. Yet the high rates of enterocyte turnover in other vertebrate species studied to date, all of which feed daily, would make it very expensive for sidewinders to maintain the intestine in a constant state of readiness.

The compromise reached is to shut down the small intestine between meals by reducing its mass and downregulating its transporters. Upon prey capture, the intestine grows in mass, its amino acid transporters are upregulated, and both are maintained at elevated levels for the duration of intestinal digestion. Rodent prey contains little carbohydrate, so the glucose transporter is upregulated for only a day or so, possibly to recover plasma glucose leaking into the intestine.

It is striking how rapid is this turn-on of the intestine, and how great is its span. Transporter activities increase within 1 day and intestinal mass within 12 h; both reach peak values around 1 day, and the resulting peak uptake capacities are 4–11 times fasting values. Because a large protein load is suddenly dumped on the sidewinder intestine, its mass is in the range for carnivorous endotherms rather than ectotherms, its transporter activities and uptake capacities for amino acids exceed even those of carnivorous endotherms and far exceed those of carnivorous ectotherms, and its specialization for amino acid transport (reflected in the P/G ratio) is extreme.

Naturally, it is costly to rebuild the sidewinder intestine. From the unprecedentedly high postfeeding surge in metabolic rate (specific dynamic action), we estimate the cost at 26% of the assimilated energy available from the prey. Still, that cost may be cheaper than the cost of maintaining the intestine uselessly ready for months. In preliminary experiments we have found even greater intestinal responses to feeding in another sit-and-wait foraging snake, the Burmese python, and much smaller responses in several actively foraging snake species that feed at frequent intervals (Secor and Diamond, unpublished data). This supports our interpretation that the remarkable hyperadaptation that we have described for sidewinder intestine is not a phylogenetic character of snakes in general but is instead a specific response to relatively large meals at long intervals.

**Outlook**

Our results raise many questions warranting further investigation, such as those that follow.

**Signals.** Hyper trophy and upregulation of the small intestine peak at a time when the ingested meal is still in the stomach and has yet to reach the intestine. What neural or hormonal signals warn the intestine that food is in the stomach?

**Other regulated functions.** Feeding triggers upregulation of intestinal brush-border transporters (this study) and pancreatic proteases (1). Are intestinal hydrolases, intestinal basolateral transporters, liver enzymes, and bile secretion similarly upregulated?

**Source for upregulation.** Because rebuilding of the intestine occurs before food reaches the intestine to be absorbed, it must be fueled by body stores that remain to be identified. Candidates include the snake’s fat bodies as a source of energy and muscle as a source of protein.

**Other snake models.** Despite the great potential advantages of rattlesnake intestine as a model for studying adaptive regulation and growth, many physiologists may hesitate because rattlesnakes are venomous. Less daunting models may be provided by nonvenomous sit-and-wait foraging snake species such as pythons, which are docile and in which the adaptive responses are even larger.
APPENDIX

We carried out validation experiments on anterior small intestines of 14 snakes, fed 4 or 5 days before death, to select the incubation time, temperature, and concentrations used for the main experiments described in the text. The rationale and methods of these validation experiments were essentially the same as those already described in detail for intestines of other vertebrate species studied previously in this laboratory (e.g., Refs. 6, 18, 20, 37, 38). Hence, we give only a brief summary of the corresponding experiments on sidewinder intestine.

Selection of Incubation Time

We incubated tissues at 30°C for 2, 4, or 8 min in solutions containing 50 mM radiolabeled L-proline, L-leucine, or D-glucose plus the appropriate radiolabeled adherent fluid marker (PEG, PEG, or L-glucose, respectively). The PEG space proved not to vary with incubation time, indicating that the adherent fluid had already equilibrated with PEG by 2 min. Calculated uptake rates (nmol·min⁻¹·mg⁻¹) were the same or nearly the same at 4 min as at 2 min for all three nutrients but declined by 50–60% at 8 min, indicating that we had by then passed beyond the linear initial phase of the uptake vs. time relation. Hence, we selected 2 min as the incubation time.

Selection of Incubation Temperature

We incubated tissues for 2 min in 50 mM L-proline or D-glucose at temperatures of 24, 30, or 35°C. The increase in uptake with temperature yielded a calculated QIo value of 1.5 for L-proline and 2.0 for D-glucose. We chose an incubation temperature of 30°C because body temperatures of free-ranging sidewinders after feeding are typically between 29 and 31°C (S. M. Secor, unpublished data).

Selection of Incubation Concentrations

We incubated tissues for 2 min at 30°C in concentrations of 0.1, 1, 50, and 100 mM L-proline or L-leucine or 0.1, 1, and 50 mM D-glucose. Total uptakes of all three solutes, and both the Na⁺-dependent and Na⁺-independent components of proline and leucine uptake, exhibited saturable kinetics. The resulting uptakes were fitted to the Michaelis-Menten equation by iterative nonlinear curve fitting to extract maximum velocity (V_max) and apparent Michaelis constant (K_m) values. For D-glucose uptake and for total uptake of L-proline and L-leucine, the resulting V_max values were 0.15, 20, and 4.6 nmol·min⁻¹·mg⁻¹, respectively, while corresponding apparent K_m values were 1.4, 37, and 5.7 mM. From 0.1 to 100 mM, the Na⁺-dependent component of L-proline uptake decreased from 34 to 20% of total uptake. For both amino acids we chose an incubation concentration of 50 mM, sufficiently far above the K_m to yield a close approximation of the V_max value for L-leucine and a modest underestimate of V_max for L-proline. (A higher incubating concentration would have reduced that underestimate, but would have had the disadvantage of requiring reducing the Na⁺ concentration considerably to maintain isotonicity.) For D-glucose we chose an incubation concentration of 20 mM, because we thereby reduced the L-glucose correction (for glucose taken up passively or in the adherent fluid) to 52% of total uptake while still remaining far above the K_m value. A D-glucose incubation concentration of 50 mM would have required an enormous L-glucose correction of 90%.

We thank Philippe Cohen for support in collecting sidewinders and John Robert, Sharon Sampogna, and Dwayne Simmons for their generous help in the measurements.

This work was supported by National Institutes of Health Grants GM-14772, DK-42973, and DK-17328 (to Univ. of California, Los Angeles, Center for Ulcer Research and Education).

References


