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Luminal nutrient signals for intestinal adaptation in pythons

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Secor, Stephen M., John S. Lane, Edward E. Whang, Stanley W. Ashley, and Jared Diamond. Luminal nutrient signals for intestinal adaptation in pythons. *Am J Physiol Gastrointest Liver Physiol* 283: G1298–G1309, 2002. First published August 28, 2002; 10.1152/ajpgi.00194.2002.—Python intestine responds rapidly to luminal nutrients by increasing mass and upregulating nutrient transport. Candidates for luminal signals triggering those responses include mechanical stimulation, single or several dietary nutrients, and endogenous secretions. To identify signals, we infused into the python's small intestine either a nonnutrient solution (saline) or a single- or multinutrient solution. Python intestine failed to respond trophically or functionally to luminal infusions of saline, glucose, lipid, or bile. Infusion of amino acids and peptides, with or without glucose, induced an intermediate response. Infusion of nutritionally complete liquid formula or natural diet induced full intestinal response. Intact meals triggered full intestinal responses without pancreatic or biliary secretions, whereas direct cephalic and gastric stimulation failed to elicit any response. Hence neither physical stimulation (cephalic, gastric, or intestinal) nor the luminal presence of glucose, lipids, or bile can induce intestinal response; instead, a combination of nutrients is required (even without pancreaticobiliary secretions), the most important being amino acids and peptides. This is understandable because pythons, as carnivores, have a high-protein diet.

digestive response; intestinal hypertrophy; intestinal nutrient transport; regulatory mechanisms

INGESTION OF A MEAL TRIGGERS a cascade of regulatory steps leading to many gastrointestinal (GI) responses. These steps, operating via luminal, secretory, hormonal, and neural signals, are mediated by the sight and smell of food, the meal's distension of the gut wall, and/or luminal contact with ingested nutrients (10, 11, 26, 30). Despite a century of research, the stimuli and mechanisms underlying GI responses remain incompletely understood. One potential source of difficulty in elucidating these regulatory mechanisms is the prevailing use in GI research of the traditional laboratory mammalian models: mice, rats, rabbits, and dogs. Because such mammals feed frequently on small meals and thus are almost constantly digesting, they characteristically express only modest regulatory spans of GI performance. To overcome this practical difficulty, we developed a new animal model, the Burmese python (*Python molurus*), to study the regulatory mechanisms of GI response (21). (Naturally, we do not assume that mechanisms in pythons, mice, and humans are identical: conclusions drawn from one species must always be tested in other species.)

Unlike laboratory mammals, pythons naturally feed at infrequent intervals (several times per year) on large meals that may exceed their own body mass (19). These feeding habits are associated with large regulatory responses (17). For example, within 24–48 h after feeding, pythons experience a 2.5-fold increase in intestinal mucosal mass, 6-fold increase in microvillus length, and up to 25- to 100-fold increases in intestinal brush-border nutrient transport, plasma hormone and lipid levels, and metabolic rate (19, 20, 22, 23). Because these factorial responses far exceed the approximately twofold responses typical of laboratory mammals, the python is an attractive model to study GI regulatory mechanisms.

Using the python model, we asked what type of proximate signal–luminal, secretory, hormonal, or neural–triggers intestinal upregulation (25). We found that intestinal segments surgically isolated from luminal nutrients and pancreaticobiliary (PB) secretions, while retaining their neural-vascular supply intact (modified Thiry-Vella loops), did not respond functionally or morphologically within 24 h after feeding (as did the intact reanastomosed intestine), but did respond functionally (by upregulating nutrient uptake) within 3 days. These results suggested that, in pythons, *1*) luminal nutrients and/or PB secretions trigger rapid functional and morphological responses; *2*) a neural and/or hormonal signal, independent of a luminal signal, can induce a delayed functional response; and *3*) the morphological and functional responses are not mechanistically coupled. Hence the logical next question is, Which specific luminal signal triggers the rapid upregulation of python intestine?

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Candidate signals include direct mechanical stimulation of the intestinal mucosa (7), a single dietary nutrient (28), a combination of dietary nutrients (27), and endogenous factors such as polyamines and gastric and PB secretions (1, 12). To identify the nutrient signal, we have now infused either a nonnutrient solution or one of six nutrient solutions directly into the small intestine via surgically placed catheters, and we have compared intestinal nutrient transport and mucosal morphology among infusion treatments. By infusing solutions downstream of the stomach and PB duct, we minimize the potential stimulatory role of gastric and PB secretions.

But there are other possible signals besides luminal nutrients. The GI tract of mammals also responds to the ultimate signals of the sight and smell of food, swallowing, and gastric distension, as well as to the proximate signals of regulatory peptides (hormones, neuropeptides, etc.); neural stimulation; and gastric, pancreatic, and biliary secretions. Although we have demonstrated an immediate response to a nutrient signal and a latent response to a hormonal or neural signal, we had not previously addressed whether any of these other agents directly stimulate the python's intestine. Snakes possess an advanced chemoreceptory system (16); hence the chemical detection of the meal may spark a cephalic-phase response of the intestine. Snakes routinely swallow prey equivalent to or exceeding the snake's own body diameter; hence the gross distension of the stomach from such a meal could mediate a gastric phase of intestinal response through neural and/or hormonal pathways. The acids, enzymes, bicarbonate, and bile secreted by the python's stomach, pancreas, and gall bladder into the proximal end of the small intestine during digestion could also trigger intestinal adaptation. Hence we tested for cephalic-phase and gastric-phase responses, respectively, by allowing snakes to capture and constrict but not swallow prey and by distending the stomach using a balloon catheter. The direct role of PB secretions on intestinal response was tested either by surgically eliminating PB secretion into the intestinal lumen or by infusing python bile directly into the small intestine.

We shall demonstrate that python small intestine *1*) does not respond to physical stimulation of the mucosa by itself nor to luminal contact with either glucose or lipids; *2*) does respond (to an intermediate degree) to luminal contact with amino acids (AA) and peptides; *3*) responds fully to luminal contact with a nutritionally complete liquid formula or natural diet; *4*) does not respond to cephalic or nonnutrient gastric stimulation alone; and *5*) does not depend on PB secretions to respond fully.

MATERIALS AND METHODS

Animals and Experimental Procedures

Animals. Burmese pythons are native to the subtropical regions of southeast Asia and feed in the wild chiefly on birds and mammals (15). They are one of the largest snake species, reaching lengths up to 6 m and masses up to 100 kg. We purchased pythons as 100-g hatchlings from a commercial breeder (Captive Bred Reptiles, Oklahoma City, OK) and maintained them in individual plastic cages on a biweekly diet of laboratory rats with water available ad libitum.

Tests for luminal nutrient signal. To test for intestinal responses to the infusion of nonnutrient and nutrient solutions (see *Infusion Solutions* for composition), we used 21 pythons [mean body mass = 751 ± 32 (SE) g], divided equally among seven infusion treatments (n = 3 per treatment). Body mass of snakes did not differ significantly among (ANOVA, P = 1.0) or between (planned pairwise comparisons, $P > 0.69$) the seven treatment groups. We surgically implanted a rubber catheter into the proximal portion of each snake's small intestine as described below. Through these catheters, we infused 12–20 ml of solution directly into each snake's small intestine at 3- to 4-h intervals. Snakes were infused over a 24-h period and received a combined volume of solution equivalent in mass to 20–30% (mean = 24.3 ± 1.1%) of the snake's body mass, thus corresponding to an average-sized natural meal. After the infusion, each snake was killed (by severing the spinal cord immediately posterior to the head), blood was drawn, and the small intestine was removed to measure mass, morphology, and nutrient uptake rates across the brush-border membrane as described in *Intestinal Nutrient Uptake.* We compared the results from these seven sets of snakes with results previously published for six pythons not subjected to any surgical procedures and killed either after a 30-day fast (n = 3) or 1 day after the ingestion of a rat meal equaling 25% of the snake's body mass (n = 3) (18). These six snakes had been bought as hatchlings from the same commercial breeder and had been maintained under identical conditions as the 21 infusion-treatment snakes. Body masses of these two sets of three snakes each did not differ (P values > 0.56) from those of the seven sets of infused snakes.

Tests for signals other than luminal nutrients. To ascertain the potential regulatory role of other signals, we undertook the following two sets of studies. First, to identify the presence of a significant cephalic phase response on the small intestine, we presented three pythons (mean mass = 999 g) with a live adult rat (weighing ~25% of the snake's body mass), which they were allowed to constrict and kill. We then removed the dead rat before the snake could attempt to swallow it, and we killed the snakes 24 h later. To assess the sole influence of gastric distension, we surgically inserted a balloon catheter (MIC bolus gastrostomy feeding tube, Ballard Medical Products, Draper, UT) into the stomach of a 530-g python. The balloon was inflated with sterile saline to simulate the distension of the stomach by a meal equivalent to 25% of the snake's body mass, and we left the balloon inflated for 24 h, after which the snake was killed.

Second, we tested the potential influence of pancreatic and biliary secretions in four pythons (mean mass = 719 g). Three pythons, one with its gall bladder surgically removed, the second with its pancreas surgically removed, and the third possessing a PB bypass, were fed rat meals equivalent to 15–26% of the snake's body mass and were killed 24 h later. The fourth python (579 g) was implanted with an intestinal catheter and infused with 2 ml of a 25% saline solution of python bile at 3-h intervals for 18 h, after which the snake was killed. The total volume of bile infused (2.4 ml) was approximately the amount released into the small intestine by a similar-size snake within 24 h of feeding. We measured from each snake its intestinal mass, morphology, and nutrient uptake rates. We compared these results with those previously published for three fasted and three fed pythons

(18), just as described above for results from snakes receiving luminal nutrient signals.

Surgical Procedures

We fasted snakes for 1 mo before surgery to ensure that the small intestine was empty (18). Snakes were anesthetized by being placed within a sealed 4-liter container containing a halothane-soaked cloth. Once anesthetized, we placed the snake on its dorsum, held it in position with cloth restraints, and scrubbed the midregion of its ventrum with Betadine solution. A 6-cm incision was made between the ventral scales and the first set of lateral scales at a site ~65% of the distance from the snout to the cloaca. The incision was retracted open to expose the pylorus, gall bladder, pancreas, and start of the small intestine.

To implant a catheter, we made a small hole in the proximal end of the small intestine distal to the junction of the PB duct, and we inserted a 10-cm rubber catheter (20 fr) through the hole. The catheter extended 2 cm downstream into the intestinal lumen and was attached to the intestine wall by a series of "purse-string" 4–0 silk sutures. We exteriorized the other end of the catheter through a small incision in the snake's body wall and sutured it to the external scales.

To remove the gall bladder or pancreas, the blood supply and ductal tissue were divided and the organ carefully excised from the surrounding tissues. We performed a PB bypass by severing the small intestine 1 cm proximal and 1 cm distal to its junction with the pancreatic and biliary ducts and then restored intestinal continuity by suturing together the segments proximal and distal to the incisions. The balloon catheter was inserted into the stomach through a 3-cm incision just proximal to the pylorus. The incision was sutured closed around the catheter with 3-0 silk, and the catheter was exteriorized through the body wall.

Incisions through the body wall were closed with an inner (muscular layer) and outer (scales) set of interrupted sutures (3-0 Vicryl; Ethicon). Immediately after surgery, we gave each snake a single dose of antibiotic (1 ml/kg enrofloxacin; Baytril Bayer) intramuscularly and continued giving injections at 3-day intervals for the next 9 days. Snakes recovered from anesthesia within 1 h and were allowed 2–4 wk of recovery before the start of experiments (except the stomach balloon experiment). During that time, snakes were provided with water ad libitum but were not fed.

Infusion Solutions

We selected seven solutions for infusion into the small intestine to determine the extent to which the intestine responds to *1*) physical stimulation alone (infusion of saline solution); *2*) a single nutrient component of a meal (carbohydrate, lipid, or AA and peptides); *3*) two different multinutrient combinations (AA and peptide-glucose or commercial liquid diet); or *4*) an approximation of a python's diet by the time it reaches the intestine (homogenized rat). Solutions were prepared as follows: saline = 0.9% NaCl intravenous solution (308 mosM; Baxter Healthcare, Deerfield, IL); carbohydrate = 58 g D-glucose (G-8270, Sigma) dissolved in 1 liter of Ringer solution (1.0 kJ/ml, final osmolarity 330 mosM); lipid = Intralipid 20% (Kabi Pharmacia, Clayton, NC), 20% lipid (soybean oil) solution manufactured for intravenous administration (8.3 kJ/ml, 324 mosM); AA and peptide = 40 g casein acid hydrolysate (80% free AA, 20% peptides; C-9386, Sigma) dissolved in 1 liter of Ringer solution (0.7 kJ/ml, final osmolarity 325 mosM); AA and peptide-carbohydrate = 23 g D-glucose and 23 g casein acid hydrolysate dissolved in 1 liter of Ringer solution (0.8 kJ/ml, final osmolarity 330 mosM); commercial liquid diet = Isocal NH (Mead Johnson, Evansville, IN) containing protein (4.2% by mass), fat (4.3%), carbohydrates (11.6%), vitamins, and minerals (4.4 kJ/ml, 270 mosM); and homogenized rat [young prekilled rats homogenized in a blender with an equal volume of saline (4.0 kJ/ml, estimate 310 mosM)]. We hereafter refer to these infusion solutions, respectively, as saline, glucose, Intralipid, AA, AA/glucose, Isocal, and homogenized rat.

Intestinal Nutrient Uptake

We measured nutrient uptake rates in vitro across the intestinal brush-border membrane as described in detail in Refs. 14 and 24. Briefly, after the severing of the spinal cord, we made a midventral incision the length of the snake and removed the small intestine. The small intestine was immediately weighed, flushed with ice-cold Ringer solution, reweighed, divided into equal-length thirds, and cut into 1-cm sleeves. Sleeves were mounted on glass rods and first incubated for 5 min in Ringer solution at 30°C and then incubated for 2 min at 30°C in a Ringer solution containing a radiolabeled nutrient and an adherent fluid marker labeled with a different radioisotope. We measured intestinal uptake rates of the AA L-aspartic acid, L-leucine, L-lysine, and L-proline (each at 50 mM and labeled with ^{3}H) and of the sugar D-glucose (at 20 mM and labeled with ^{14}C). Each of the four AA is transported predominantly by one of the major intestinal AA carriers: the acidic, neutral, basic, and imino acid transporter (29). To correct for the amount of radiolabeled nutrient in the fluid adherent to the intestine, we used a second labeled solute: ^{14}C-polyethylene glycol for the AA, and L-[^{3}H]glucose for D-glucose. L-[^{3}H]glucose also corrects for D-glucose transported by passive diffusion. Hence, after these corrections, our measurements yield total uptake (carrier-mediated plus passive) of each AA and carrier-mediated uptake of D-glucose. We express uptake rates as nanomoles per minute per milligram of sleeve wet mass. We calculated nutrient uptake capacity (μmol/min) of the whole length of the small intestine by multiplying uptake rate ($nmol \cdot min^{-1} \cdot mg^{-1}$) times wet mass (mg) for each of the equal-length thirds and then summing these three products.

Intestinal Morphology

We assessed the morphological response of the small intestine to each of the infusion treatments in the following five ways. First, we weighed each snake's intact small intestine flushed of its contents. Second, for each snake, we weighed and averaged the wet masses of five individual 1-cm sleeves taken from each intestinal segment. Third, using the wet and dry masses of scrapeable mucosa and residual serosa of 1-cm sleeves taken from each intestinal segment, we calculated each snake's total small intestinal mucosal and serosal wet and dry masses. Fourth, we used standard light microscopy techniques to measure enterocyte height and width for the proximal intestine. A single 1-cm sleeve from the proximal segment was fixed in 10% neutral-buffered formalin solution, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. For each snake, we averaged the height and width (measured using an optical micrometer) and calculated the volume (based on the formula for a cylinder) of five enterocytes.

Fifth, we used electron microscopy to measure microvillus length of proximal intestinal segments. Small samples of brush-border membrane were fixed in 4% glutaraldehyde solution, postfixed with 1% osmium tetroxide, dehydrated in a graded series of ethanol, embedded in plastic, sectioned at 90 nm, stained with uranyl acetate and lead, and examined

with a JEOL JEM-100 CX electron microscope. For each snake, one representative sample was selected, and four or five regions of microvillus border were photographed at a magnification of ×7,200. From each of those four or five prints, we measured the length of 10 microvilli, using only microvilli cut in the central plane parallel to their long axis. Actual microvillus length was calculated by dividing the measured length from the print by total print magnification (×21,600) and then averaged for each snake.

Morphological Response of Other Organs

We previously documented postprandial changes in the masses of several organs for pythons that ingested intact rodent meals (18, 20). Hence in this study, we assessed the effects of each of the seven infused solutions on the wet and dry masses of the heart, lungs, liver, stomach, pancreas, gall bladder, large intestine, and kidneys. Each organ was weighed immediately on its removal from the snake (wet mass), dried at 60°C for 14 days, and reweighed (dry mass).

Blood Chemistry

Because pythons experience postprandial changes in blood chemistry (18, 20), we tested for such changes after each infusion treatment. Whole blood (2–3 ml) was drawn from each snake's heart immediately after the severing of its spinal cord and centrifuged for 10 min at 4,000 revolutions/min at 4°C. Plasma was analyzed (Hitachi blood analyzer, model 747) for concentrations of Na^+, K^+, Cl^-, Ca^{2+}, phosphorus, CO_2, glucose, creatinine, total protein, and albumin.

Statistical Analyses

We used a repeated-design ANOVA to test for positional effects (proximal, middle, distal) on nutrient uptake rates for each of the seven infusion treatments. An ANOVA was employed to test for significant effects of infusion treatments on intestinal uptake rates and blood chemistry. Analysis of covariance (ANCOVA), with body mass as a covariate, was used to test for treatment effects on small intestinal uptake capacities, intestinal morphology, and organ masses. In conjunction with ANOVA or ANCOVA, we made a priori planned, pairwise, mean comparisons between pairs of treatments. We repeated all of these analyses after including data from the three fasted and three fed snakes reported previously (18). Similarly, we used ANOVA or ANCOVA to assess differences between fasted and fed snakes and snakes tested for cephalic and PB signals. We provide *P* values for ANOVAs, ANCOVAs, and significant pairwise comparisons. We take the level of statistical significance as $P < 0.05$, and we report mean values as means ± 1 SE. We conducted all statistical analyses by the microcomputer version of SAS.

RESULTS

Tests of Luminal Nutrient Signal

Intestinal nutrient uptake. POSITIONAL EFFECTS. We detected no significant effects of intestinal position on uptake rates for 25 of the 35 solute-treatment combinations (seven infusions multiplied by uptakes of five solutes). For the remaining 10 combinations, uptake rates of the proximal and middle segments were statistically indistinguishable, with either one or both of those segments possessing significantly higher uptake rates than the distal segment. We, therefore, averaged uptake rates from the proximal and middle segments and, henceforth, refer to this intestinal region as the anterior small intestine.

ANTERIOR SMALL INTESTINE UPTAKE RATES. Uptake rates of all five measured solutes by the anterior small intestine differed significantly (ANOVA, *P* values <0.015) among the seven infusion treatments. In general, solute uptake rates in response to the infusion of saline, glucose, or Intralipid did not differ and were significantly less than for intestines infused with either AA or AA/glucose solution (Fig. 1). Intestines infused with either Isocal or homogenized rat possessed significantly greater (*P* values <0.05) uptake rates than intestines of the other five treatments. Compared with

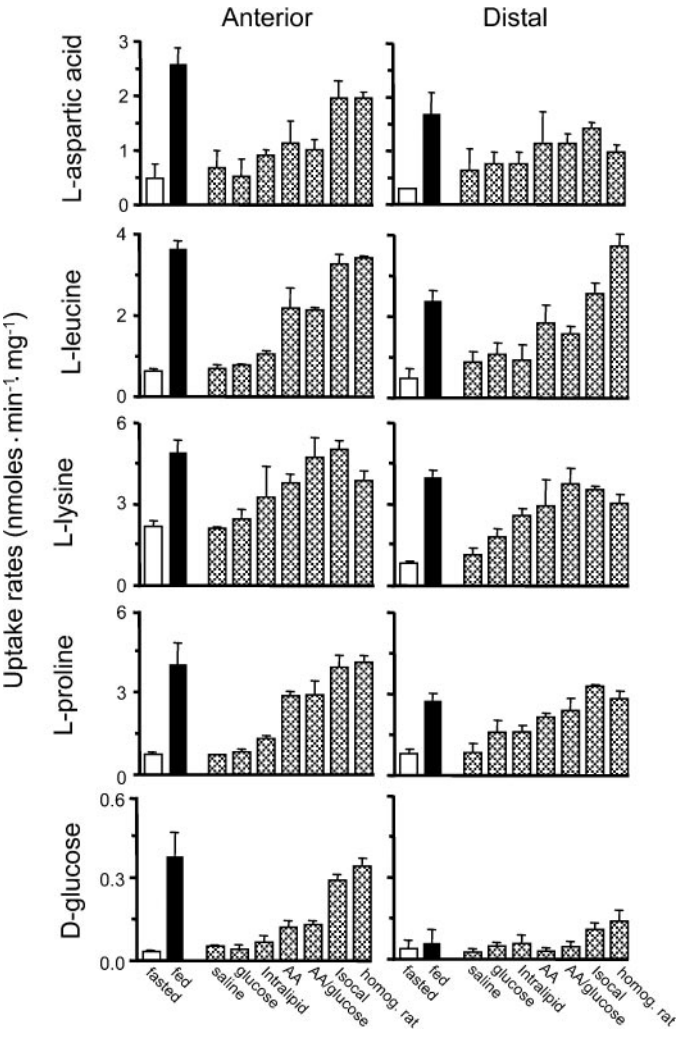

Fig. 1. Brush-border uptake rates of the solutes L-aspartic acid, L-leucine, L-lysine, L-proline, and D-glucose by the anterior and distal regions of the small intestine of pythons infused intestinally with 1 of the 7 following solutions: saline, glucose, Intralipid, amino acids (AA), AA/glucose, Isocal, or homogenized rat. For comparison, we show uptake rates of fasted pythons (open bars) and of fed pythons 1 day after consuming an intact rodent meal equaling 25% of the snake's body mass (solid bars). Values are means ± 1 SE; sample size = 3 snakes. Note that snakes infused with saline, glucose, or Intralipid yield uptake rates no higher than those of fasted snakes; snakes infused with Isocal or homogenized rat yield uptakes as high as those of fed snakes; and snakes infused with AA or AA/glucose yield intermediate uptakes.

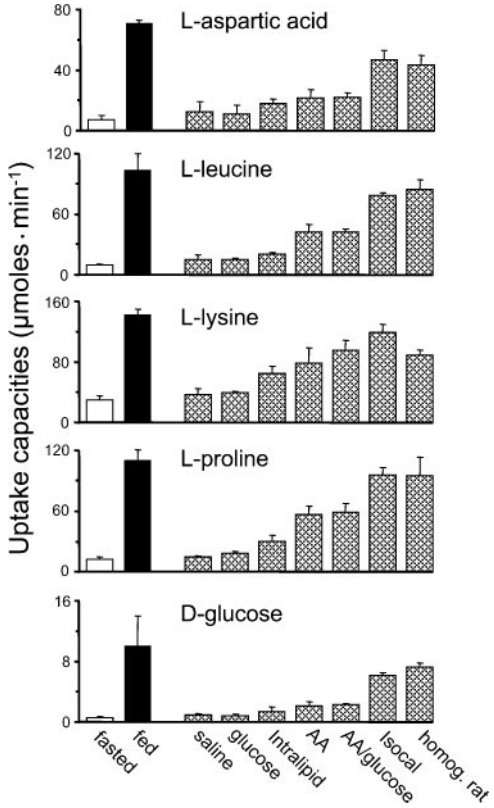

Fig. 2. Solutes and solutions are as described in Fig. 1. Uptake capacities (μmol/min) are illustrated instead of uptake rates. Values are means ± 1 SE; sample size = 3 snakes. Note that snakes infused with saline, glucose, or Intralipid yield uptake capacities no higher than those of fasted snakes; snakes infused with Isocal or homogenized rat yield capacities as high as those of fed snakes; and snakes infused with AA or AA/glucose yield intermediate uptake capacities

fasted snakes, snakes infused with saline, glucose, or Intralipid did not differ significantly in anterior uptake rates for any of the five solutes. Snakes infused with Isocal or homogenized rat yield uptake rates of the five nutrients not different from those of fed snakes 1 day after the ingestion of an intact meal.

DISTAL SMALL INTESTINE UPTAKE RATES. As true of anterior small intestine, transport rates of each solute by the distal small intestine demonstrated significant (P values <0.03) variation among infusion treatments, except for aspartic acid (Fig. 1). Distal uptakes of solutes other than aspartic acid were lowest for intestines infused with saline, glucose, or Intralipid; intermediate for intestines infused with AA or AA/glucose; and highest for intestines infused with Isocal or homogenized rat. Distal uptakes of snakes infused with saline, glucose, or Intralipid did not differ from those of fasted snakes. Distal uptakes of snakes infused with AA, AA/glucose, Isocal, or homogenized rat did not differ from those of fed snakes 1 day after ingestion of an intact meal.

NUTRIENT UPTAKE CAPACITIES. Total small intestinal uptake capacity (summed products of regional uptake rates times intestinal wet mass) differed significantly (P values <0.002) among the seven infusion treatments for all five solutes (Fig. 2). Capacities did not differ among intestines infused with saline, glucose, or Intralipid. Capacities tended to be significantly higher after the infusion of AA or AA/glucose and were further elevated with the infusion of Isocal or homogenized rat. For example, the infusion of Isocal generated uptake capacities three- to eightfold greater than after the infusion of glucose. This increase in capacity results from the product of two increases: the (on average) 400% increase in mass-specific uptake rates described in ANTERIOR SMALL INTESTINE UPTAKE RATES and DISTAL SMALL INTESTINE UPTAKE RATES above and the increase in small intestinal mass to be described in *Intestinal morphology* below. With the use of fasted snakes and fed snakes 1 day after feeding as benchmarks, there were no significant differences in capacities between fasted snakes and snakes infused with saline, glucose, or Intralipid; there were no differences in capacities between fed snakes and snakes infused with Isocal or homogenized rat (with the exception of aspartic acid); and capacities of snakes infused with AA or AA/glucose were intermediate.

Intestinal morphology. INTESTINAL MASS. Small intestinal mass differed significantly ($P = 0.022$) among infusion treatments, becoming significantly (P values <0.05) heavier in snakes infused with Intralipid, Isocal, or homogenized rat than in snakes infused with either saline or glucose (Fig. 3*A*). Compared with fasted intestines, intestines infused with glucose, saline, AA, or AA/glucose solutions did not significantly differ in mass, whereas intestines infused with In-

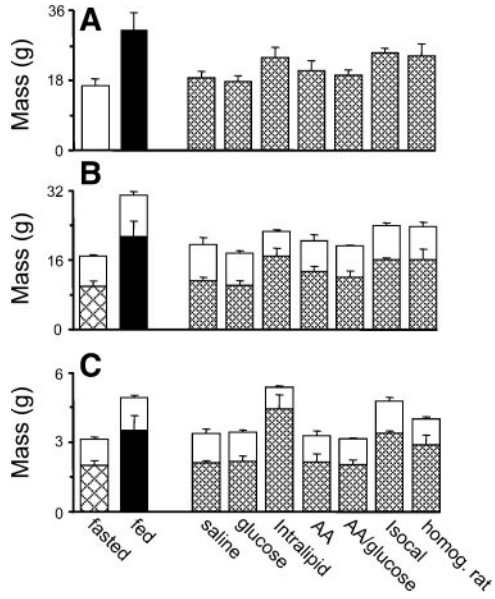

Fig. 3. Small intestinal wet mass ("whole intestine"; *A*); mucosal (fine cross-hatched bars) and serosal (open bars) wet mass (*B*); and mucosal (fine cross-hatched bars) and serosal (open bars) dry mass (*C*) of pythons infused with 1 of 7 solutions. Intestinal wet mass (*A*) and mucosal (*B* and *C*; wide cross-hatched or solid bars) and serosal (*B* and *C*; open bars) wet and dry masses of fasted pythons and fed pythons 1 day postfeeding are presented for comparison. Values are means ± 1 SE; sample size = 3 snakes. Note the elevated mass response of whole intestine and mucosa of snakes infused with Intralipid, Isocal, and homogenized rat, compared with fasted snakes or with snakes receiving the other infusions.

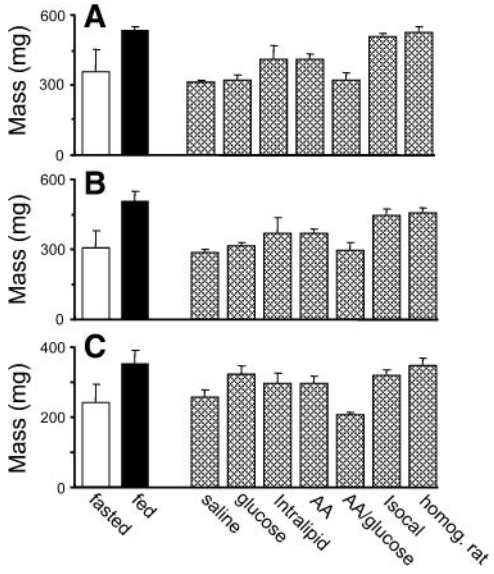

Fig. 4. Wet masses of 1-cm segments taken from the proximal (*A*), middle (*B*), and distal (*C*) regions of the small intestine of pythons infused with 1 of 7 solutions. Segment masses of each region from fasted pythons (open bars) and fed pythons 1 day postfeeding (solid bars) are presented for comparison. Values are means ± 1 SE; sample size = 3 snakes. Note that the mass for pythons infused with either Isocal or homogenized rat is similar to that for fed pythons 1 day postfeeding.

tralipid, Isocal, or homogenized rat were significantly (P values <0.032) heavier by 44–50%. Small intestinal mass of fed snakes 1 day after consuming an intact meal was significantly (P values <0.032) greater than that found for any infusion treatment.

INTESTINAL MUCOSA AND SEROSA. Wet and dry masses of the intestinal mucosa varied significantly (P values <0.003) among infusion treatments, but wet and dry masses of the serosa did not (Fig. 3, *B* and *C*). Mucosal wet masses of snakes infused with AA, Intralipid, Isocal, or homogenized rat were significantly (P values <0.05) heavier (by 20–66%) than those of snakes infused with saline or glucose solutions (Fig. 3*B*). Mucosal dry mass of snakes infused with Intralipid was significantly (P values <0.02) heavier than for any other infusion treatment and was heavier (P values <0.05) for snakes infused with Isocal or homogenized rat than for snakes infused with saline and AA/glucose solutions (Fig. 3*C*). Intestinal mucosa of fed snakes digesting intact rats was significantly (P values <0.026) greater in wet mass than for any of the infusion-treatment snakes and greater (P values <0.039) in dry mass than for snakes infused with saline, glucose, AA, or AA/glucose. Mucosal wet and dry masses of fasted intestine were similar to those of snakes infused with saline, glucose, or AA/glucose solutions.

INTESTINAL SEGMENTS. Mass of 1-cm segments declined from the proximal to the distal end of the small intestine. For each intestinal region (proximal, middle, and distal), segment mass differed significantly (P values <0.02) among infusion treatments (Fig. 4). Segments of each region infused with Intralipid, Isocal, or homogenized rat were significantly (P values <0.05) heavier than segments infused with saline or AA/glucose solutions. AA infusion resulted in a 30% increase (P values <0.05) in the mass of proximal segments compared with the infusion of saline or glucose. Segment masses were similar between fed snakes digesting intact rodents and snakes infused with Intralipid, Isocal, or homogenized rat. Segment masses of fasted snakes did not differ from those of snakes infused with saline, glucose, AA, or AA/glucose.

ENTEROCYTE MORPHOLOGY. Enterocyte height, width, and volume differed significantly (P values <0.035) among the seven infusion treatments (Fig. 5). Compared with enterocytes from saline-infused intestine, cells were significantly shorter after the infusion of AA or AA/glucose and significantly wider after the infusion of Intralipid, AA, or homogenized rat. Enterocyte volume was greatest after the infusion of Intralipid and homogenized rat and more than double the volume of cells exposed to saline. Enterocyte sizes were comparable among fasted snakes and snakes infused with saline, glucose, AA, or AA/glucose and similar among snakes digesting intact meals and those infused with Intralipid or homogenized rat.

MICROVILLUS LENGTH. Length of intestinal microvilli differed significantly ($P = 0.004$) among infusion treatments (Fig. 6). Microvilli of snakes infused with homogenized rat were significantly (P values <0.044) longer than for any other infusion treatment, being three times longer than the shortest microvilli, those from saline-infused intestines. Microvilli of intestines infused with AA or Isocal were significantly longer than those of intestines infused with saline. Fed snakes digesting an intact meal had longer microvilli

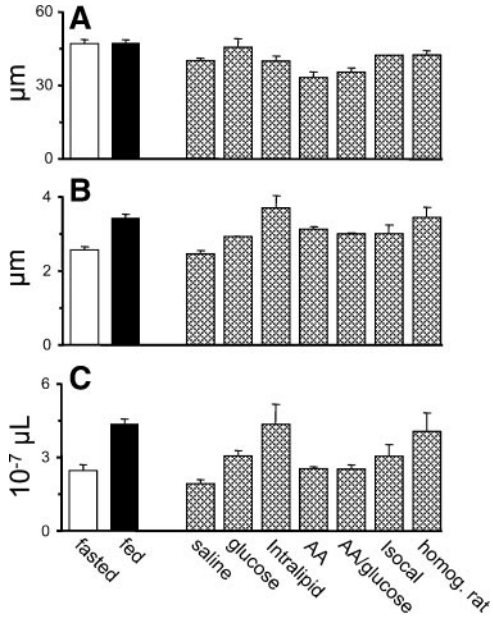

Fig. 5. Enterocyte height (*A*), diameter (*B*), and volume (*C*) from the proximal intestine of pythons infused with 1 of 7 solutions and of fasted pythons (open bars) and fed pythons 1 day postfeeding (filled bars). Values are means ± 1 SE; sample size = 3 snakes. Note that the enlargement of enterocytes exposed to the infusion of Intralipid or homogenized rat is similar to that of fed snakes.

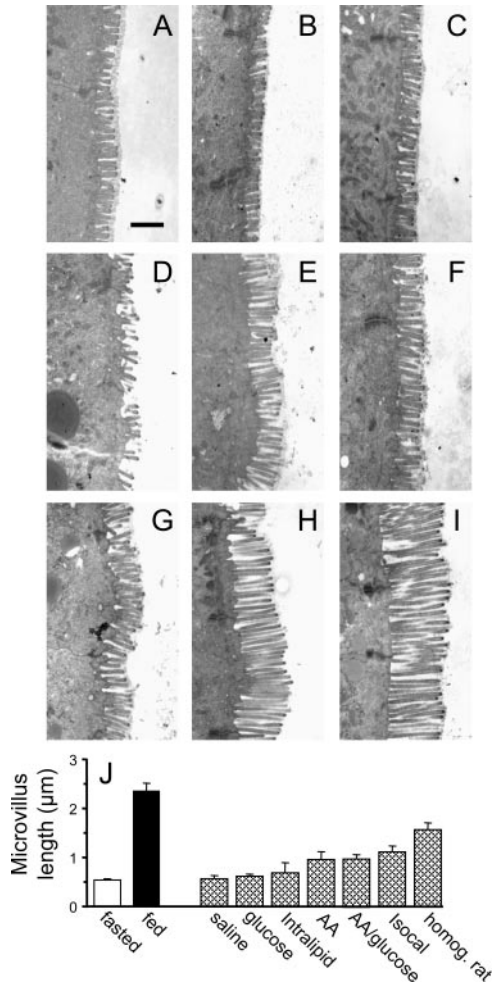

Fig. 6. Electron micrographs of proximal intestinal microvilli from fasted pythons (*A*); from pythons infused with saline (*B*), glucose (*C*), Intralipid (*D*), AA (*E*), AA/glucose (*F*), Isocal (*G*), or homogenized rat (*H*); and from fed pythons 1 day postfeeding (*I*). *J*: microvillus length for each treatment. Values are means ± 1 SE; sample size = 3 snakes. Bar in *A* represents 1 μm. Note that microvilli of snakes infused with saline, glucose, or Intralipid are as short as those of fasted snakes; that microvillus length of snakes infused with AA, AA/glucose, Isocal, and homogenized rat is intermediate; and that fed snakes have the longest microvilli.

than snakes receiving any of the infusion treatments, whereas microvilli of intestines infused with saline, glucose, Intralipid, or AA/glucose were as short as those of fasted snakes.

Masses of other organs. Only kidney wet mass varied significantly ($P = 0.048$) among the seven infusion treatments, being significantly (P values <0.05) heavier (by 40%) in snakes infused with AA, Isocal, or homogenized rat than in snakes infused with saline (Fig. 7). Wet and dry masses of the liver, stomach, and pancreas, as well as the kidney wet mass, were significantly greater in fed snakes 1 day after ingesting intact meals than in fasted snakes or in any of the infused snakes (Fig. 7). This trend was reversed for the wet and dry masses of the gall bladder, including its contents (significantly less in fed snakes than in any other snakes). Organ masses did not differ between fasted snakes and snakes of any infusion treatment.

Blood chemistry. Plasma concentrations of Na^+ ($P = 0.004$), K^+ ($P = 0.006$), Cl^- ($P = 0.010$), CO_2 ($P = 0.006$), creatinine ($P = 0.012$), albumin ($P = 0.043$), and phosphorus ($P = 0.004$) varied significantly among infusion treatments, whereas concentrations of Ca^{2+} ($P = 0.086$), glucose ($P = 0.511$), and protein ($P = 0.060$) lacked significant treatment response (Fig. 8). Snakes infused with saline, glucose, or homogenized rat tended to possess significantly higher plasma levels of Na^+, K^+, and Cl^- than snakes infused with Intralipid, AA, or AA/glucose solutions, with the most striking difference being the ~30 mM higher Na^+ and Cl^- concentrations of snakes infused with saline compared with snakes infused with Intralipid. Plasma CO_2 of snakes infused with either Intralipid or glucose was three times higher (P values <0.004) than for snakes infused with either homogenized rat or saline. Snakes infused with homogenized rat or saline possessed significantly (P values <0.03) higher plasma creatinine concentrations than other infused snakes and higher ($P < 0.013$) phosphorus levels than snakes infused with either AA or AA/glucose solution. Plasma solute concentrations of fasted snakes and of fed snakes 1 day after ingesting an intact meal were generally within

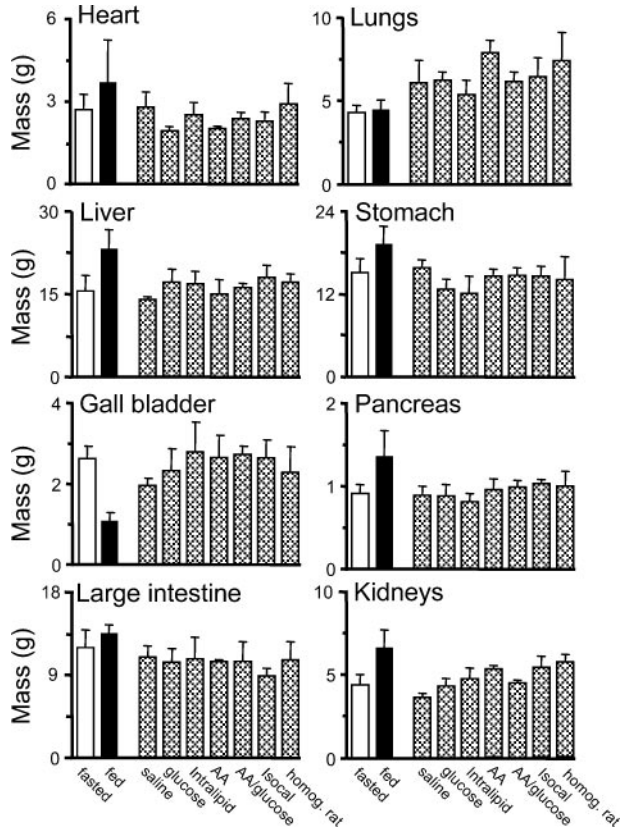

Fig. 7. Wet masses of the heart, lungs, liver, stomach, full gall bladder, pancreas, large intestine, and kidneys of pythons infused intestinally with 1 of 7 solutions. Wet masses of each organ from fasted pythons (open bars) and fed pythons 1 day postfeeding (solid bars) are presented for comparison. Values are means ± 1 SE; sample size = 3 snakes. Note the general lack of significant variation of organ wet masses among infusion treatments with the exception of the kidneys. Organ dry masses yielded similar conclusions.

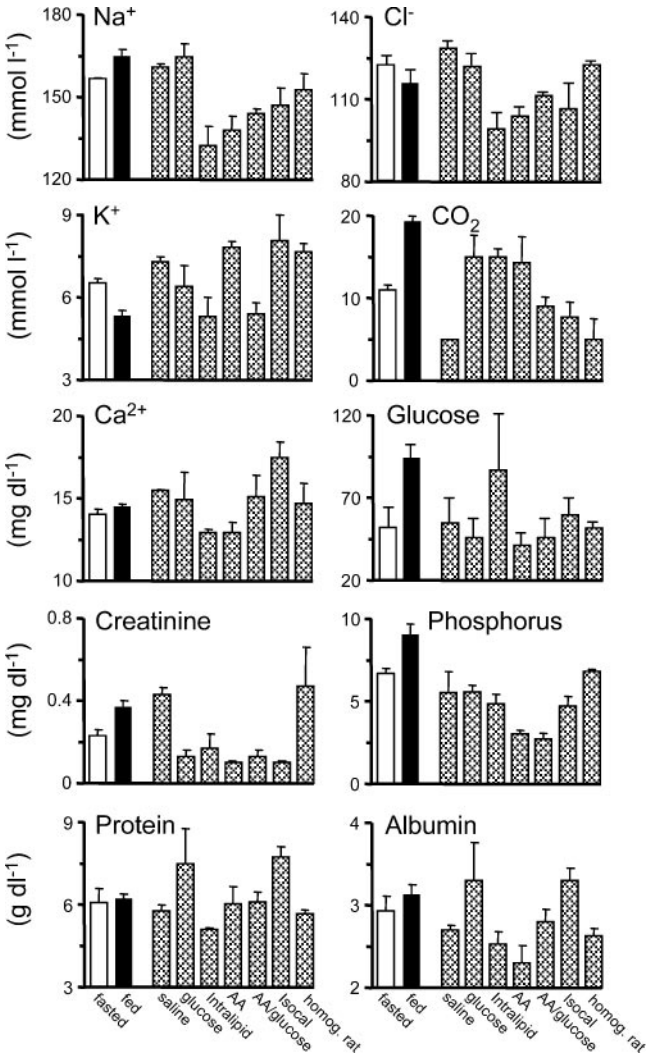

Fig. 8. Plasma concentrations of 10 solutes in pythons infused with 1 of 7 solutions and in fasted pythons (open bars) and pythons 1 day postfeeding (solid bars). Values are means ± 1 SE; sample size = 3 snakes. Note the significant variation among infusion treatments in Na^+, Cl^-, K^+, CO_2, creatinine, phosphorus, and albumin concentrations.

the range of values found for infused snakes, except for the significantly (P values <0.02) higher phosphorus levels of fed snakes.

Tests of Signals Other Than Luminal Nutrients

Cephalic and gastric stimulation. INTESTINAL NUTRIENT UPTAKE. Snakes that constricted and killed prey, but that were not allowed to swallow the prey ("cephalic stimulation"), exhibited low rates of intestinal nutrient uptake (Fig. 9). For all cases except for anterior intestinal glucose and distal intestinal lysine uptake, uptake rates by the anterior and distal small intestine of snakes stimulated only by cephalic interaction with the meal did not differ significantly from those of fasted snakes, while being significantly (P values <0.039) less than uptake rates (both anterior and distal measures) of fed snakes 1 day after ingesting an intact meal. Similarly, intestinal uptake capacities of snakes stimulated by meal constriction alone did not differ from those of fasted snakes and were significantly (P values <0.037) less than capacities of fed snakes. Intestinal solute uptake of the snake whose stomach was distended by a balloon catheter ("gastric stimulation") more closely matched rates characteristic of fasted snakes than fed snakes (Fig. 9). Averaged across solutes, uptake capacities of this snake were only 23% of the capacities of fed snakes 1 day after the ingestion of an intact meal.

INTESTINAL MORPHOLOGY. Small intestinal masses of cephalic-stimulated snakes were found to be similar to those of fasted snakes and significantly ($P = 0.0009$) less than those of fed snakes (Fig. 10*A*). Similarly, 1-cm intestinal segments for each intestinal region did not differ significantly in mass between fasted snakes and snakes stimulated only by meal constriction (Fig. 10, *B*, *C*, and *D*). Total intestinal mass and microvillus length

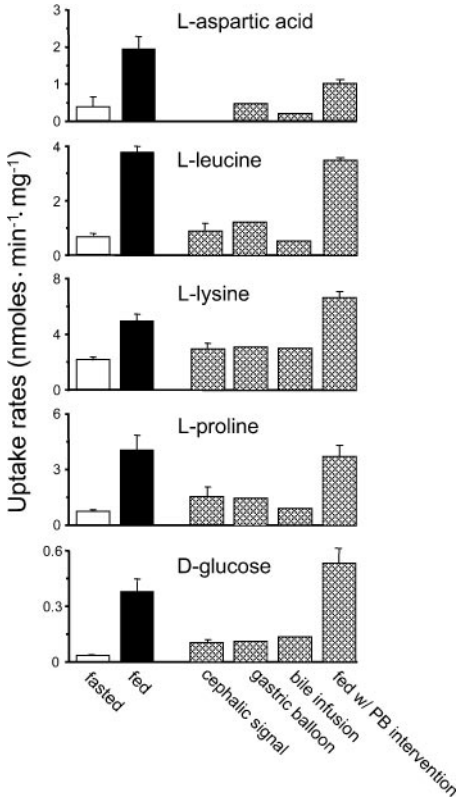

Fig. 9. Brush-border uptake rates of L-aspartic acid, L-leucine, L-lysine, L-proline, and D-glucose by the anterior region of the small intestine of pythons under the following conditions: 1 day after the constriction of rodent meals but no ingestion (cephalic signal, $n = 3$); after 1 day of the stomach being stretched by a balloon catheter to simulate the distension of the stomach wall by a rodent meal (gastric balloon, $n = 1$); after 1 day of the proximal intestine being infused with python bile (bile infusion, $n = 1$); and 1 day after the ingestion of a rodent meal that was 15–25% of body mass for pythons lacking a pancreas, lacking a gall bladder, or possessing a pancreaticobiliary duct bypass (fed w/ PB intervention, $n = 3$). Nutrient uptake rates of fasted pythons (open bars, $n = 3$) and fed pythons 1 day postfeeding (solid bars, $n = 3$) are presented for comparison. Values are means ± 1 SE. Note the lack of upregulation of intestinal nutrient uptake for the cephalic signal, gastric balloon, and bile infusion treatment, and full upregulation in fed pythons lacking intestinal pancreatic and/or biliary secretions.

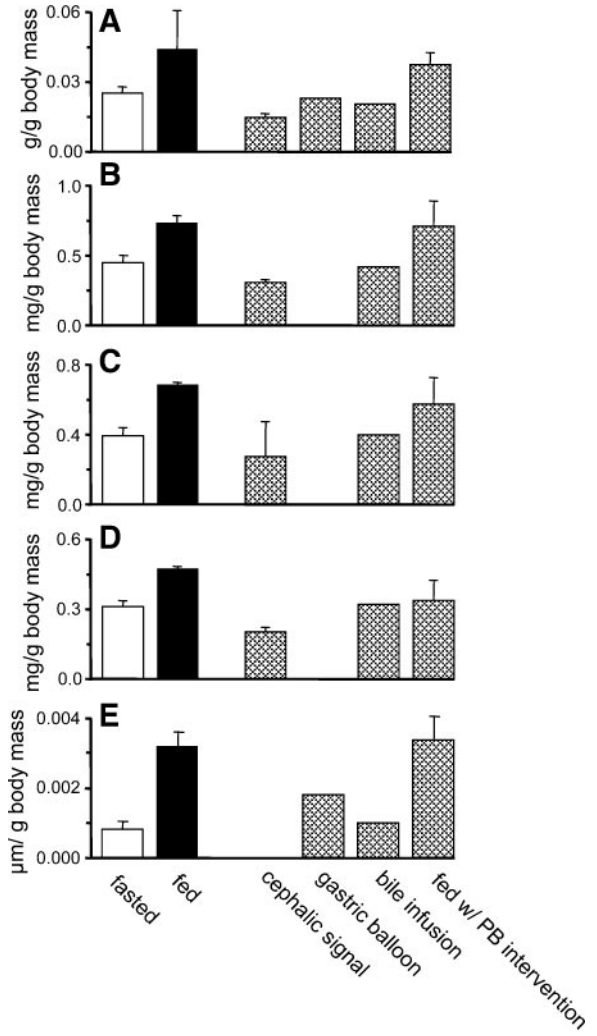

Fig. 10. Small intestinal mass (*A*), mass of proximal (*B*), middle (*C*), and distal intestinal segments (*D*), and length of proximal intestinal microvilli (*E*) of pythons following the treatments described in Fig. 9. Because of the twofold range in body mass among treatments, morphological measures are presented normalized to body mass. Values are means ± 1 SE. Note the lack of a morphological response after cephalic and gastric signaling and the intestinal infusion of bile, whereas pythons 1 day after ingesting intact meals but lacking pancreatic and/or biliary secretions exhibit full morphological responses.

of the snake whose stomach was distended for 24 h with a balloon catheter were within the range of values observed for fasted snakes (Fig. 10) and were lower than values of fed snakes.

PB secretions. INTESTINAL NUTRIENT UPTAKE. Removing or bypassing the gall bladder and/or pancreas had no apparent effects on the upregulation of intestinal nutrient uptake after the ingestion of an intact meal (Fig. 9). Uptake rates of all solutes by the anterior and distal small intestine did not differ 1 day after the ingestion of a rodent meal between snakes possessing intact tissues and snakes whose PB secretions were reduced or diverted. Intestinal uptake capacities of PB-altered snakes were similar to those of fed snakes (except for aspartic acid) and significantly greater (P values <0.05) than those of fasted snakes. The infusion of bile for 18 h apparently failed to induce upregulation of solute transport: transport rates of all solutes remained at fasting levels (Fig. 9).

INTESTINAL MORPHOLOGY. Total intestinal mass, mass of 1-cm intestinal segments, and microvillus length of snakes whose gall bladder and/or pancreas were removed or bypassed did not differ from those of fed snakes 1 day after swallowing an intact meal (Fig. 10). In contrast, these intestinal variables of the snake infused with bile were comparable to those of fasted snakes, indicating an apparent lack of a morphological response to bile infusion (Fig. 10).

DISCUSSION

A conclusion of our earlier study (25) was that a luminal signal, either luminal nutrients and/or PB secretions, is responsible for the rapid postprandial responses of the python's small intestine. To identify whether a specific luminal nutrient signal triggers those rapid responses, we have now tested the intestine's responses to the direct infusion of saline, single nutrients, or combination of nutrients. The resulting responses ranged from no observable response (after the infusion of saline or glucose) to a full suite of responses (after the infusion of homogenized rat). The luminal infusion of lipids (Intralipid) elicited no upregulation of nutrient uptake, and we explain below why we conclude that the apparent effect of lipids on intestinal growth is not real. The introduction of an AA solution, with or without glucose, elicited moderate upregulation of nutrient uptake and of microvillus length but had little or no other effect on intestinal growth. Only the infusion of a commercial multinutrient liquid diet (Isocal) or of homogenized rats caused the python's intestine to respond in a pattern consistent with that following the ingestion of an intact rodent meal. Hence it appears that a combination of luminal nutrients is required to stimulate full intestinal upregulation and mucosal growth, and that the most important component is AA and peptides. In addition, there is no apparent cephalic-phase response of the small intestine triggered by capturing and constricting of prey, no apparent gastric-phase response triggered by distention of the gastric wall, and no evidence that PB secretions are necessary to trigger intestinal adaptation.

We shall now discuss the intestine's response to mechanical and nutrient stimulation, address other potential signals of intestinal response, and conclude by proposing four projects to further investigate the regulatory role of nutrient and hormonal signals in triggering intestinal adaptation using the python model.

Mechanical and Nutrient Stimulation of Intestinal Responses in Pythons

Mechanical stimulation. We infused a nonnutrient saline solution to stimulate the intestine mechanically by causing movement and expansion of the mucosa.

Although such physical luminal stimulation produced a growth response of rat intestinal Thiry-Vella loops (7), in pythons it did not do so, nor did it cause upregulation of nutrient uptake. We can also conclude that the absorbed ions from the saline solution, although reflected in increased plasma concentrations of Na^+ and Cl^- (Fig. 8), failed to trigger intestinal response in the python, just as they failed to do so in rats (6) and dogs (2).

Single nutrients. Luminal glucose is well documented in mammals to stimulate mucosal growth and glucose uptake (9). The lack of any observable response to glucose infusion of the python may have two explanations. First, whereas the mammals that have demonstrated a response to luminal glucose are herbivores or omnivores (rodents and dogs), pythons are strict carnivores and may, therefore, not have evolved any regulatory response to glucose because there is so little of it in their diet. Second, the mammalian response appears only at high, infused glucose concentrations (27). The glucose solution (5.8%) that we found ineffective in pythons is close in concentration to the 5% glucose solution found to be ineffective in mammals (27). We decided not to infuse a more concentrated glucose solution into pythons out of concern that its hypertonicity might result in mucosal damage, impairment of nutrient transport, and dehydration (13).

On first examination, the infusion of Intralipid, produces a contrasting set of responses: no upregulation of nutrient uptake (Fig. 1), but noticeable increase in mucosal mass (Fig. 3) and enterocyte volume (Fig. 5*C*). On closer inspection, however, the increases in mucosal mass and enterocyte volume may just be due to the enterocytes becoming filled with lipid droplets because of rapid diffusion of the infused lipids into the enterocytes. There is no evidence of growth of the cells apart from their lipid content: for instance, their microvilli underwent no increase in length (Fig. 6). Hence python intestine apparently does not respond either functionally or trophically to intraluminal lipids, even at a concentration of 20% and a caloric value of 8.3 kJ/ml.

In contrast to glucose and lipids, infusion of protein hydrolysate (80% free AA, 20% peptides) stimulated both functional and morphological responses of the python's intestine. Intraluminal AA, like glucose, can stimulate mucosal growth and AA uptake in mammals at AA concentrations as low as 5% (8). The 4% AA solution that we infused is apparently sufficient, even at a caloric content of 0.7 kJ/ml, to stimulate an intermediate level of nutrient transport and growth. Either higher AA concentrations or else other additional nutrients are necessary to spark complete adaptation.

Nutrient combinations. An equal mixture (by weight) of AA/peptides and glucose induced intestinal responses almost identical to those induced by AA/peptide alone. This suggests that glucose and AA/peptide do not act synergistically to elevate intestinal responses. If glucose has no stimulatory role at all, then we can also conclude that python intestine responds to an AA concentration as low as 2.3%. The commercial liquid diet Isocal, which is designed to provide a balance of carbohydrates, fats, proteins, vitamins, and minerals for human nutrition, elicited full upregulation of intestinal function and morphology in the python. Experimental infusion of similar balanced synthetic diets similarly stimulated mucosal growth, intestinal motility, and disaccharidase activity in mammals (2, 3, 27). Because pythons are carnivores, it is not surprising that the infusion of homogenized rat triggered the full natural complement of intestinal responses.

Signals Other than Luminal Nutrients Triggering Intestinal Responses

We found that a cephalic signal generated by the sight, smell, taste, and contact with a live meal and a gastric signal generated by a mechanical, nonnutrient distention of the stomach wall were unable to trigger morphological or functional responses of the python's small intestine. Pythons respond metabolically to the capture and constriction of prey (but not swallowing) by elevating oxygen consumption rates by 23% within 12 h, with rates returning to baseline values within 72 h (20). The magnitude of this response is trivial compared with the 1,600% increase in oxygen consumption rates that the snakes would have experienced had they been allowed to swallow the prey (18). We conclude from these findings that, whereas some cephalic-phase and gastric-phase responses may exist as anticipatory signals in pythons, these signals alone cannot trigger intestinal adaptation, which requires at least the ingestion of a multinutrient meal and may further require the passage of that meal into the small intestine.

PB secretions are, of course, important to python digestion, but not as trophogenic agents. Although proposed as trophogenic agents (1), these secretions are apparently not necessary to trigger the postprandial adaptation of the python intestine. The findings from the four pythons used to study the role of PB secretions are in accordance with results of the nutrient infusion study. The infusion of nutrients downstream of the PB duct did not appear to stimulate either the gall bladder or pancreas to secrete. This was evident by the lack of the characteristic postprandial reduction in gall bladder mass (usually by 60%) and increase in pancreatic mass (usually by 50%) occurring within 24 h (18, 20). The former reduction results from stored bile being released into the intestine, and the latter increase is a response to upregulate pancreatic capacity to secrete enzymes and bicarbonate.

Further Studies of Regulatory Signals in the Python Model

The present study and our previous study (25) make clear that python small intestine responds rapidly to ingested nutrients and that AA/peptides play a disproportionate role in this response. The greater response to AA/peptides than to glucose or lipid may be explained simply by the fact that a python's natural meal (live wild birds and mammals) consists largely of pro-

tein, with much less fat and very little carbohydrate. Despite this importance of luminal signals, we should not dismiss the possible role of neural, hormonal, and/or paracrine signal(s) as well. Our finding of transporter upregulation in isolated intestinal loops of fed pythons suggests a neural or hormonal regulatory pathway. A possible mechanistic base for such a pathway is provided by our observation of the postprandial release of four GI peptides into circulation and our identification of six other GI peptides within the python's GI tract (22). Advantages of the python model for studies of gut regulation include its large regulatory spans, the ease with which surgical procedures can be carried out on pythons, and the disassociation in pythons between functional and trophic responses (21, 25). Although the regulatory mechanisms of pythons and mammals will inevitably exhibit differences, studies using the python model could facilitate the discovery of corresponding mechanisms in mammals, just as studies of squid axon facilitated the design of studies of mammalian nerves. Hence we propose the following four experiments, using the python model, to explore further the regulatory mechanisms of digestion.

Nutrient response of isolated intestinal loops. We have found that Thiry-Vella loops can be easily constructed in pythons and that python intact intestine responds fully to luminal infusion of a nutritionally complete liquid diet. Hence a logical next step would be to infuse a liquid diet into Thiry-Vella loops, to assess whether these isolated loops respond trophically and/or functionally to luminal nutrients.

Upstream or downstream induction of intestinal adaptation. Mammalian duodenum and jejunum respond morphologically and functionally to ileal infusions of nutrients (6, 31). Would nutrient infusion into one region of intact python intestine trigger adaptation in another region that has no contact with nutrients? Such experiments could help identify the anatomic locus of regulatory signals.

Regulation of intestinal function by GI regulatory peptides. The functional upregulation observed, after pythons have fed, in intestinal loops isolated from luminal nutrients, suggests a neural or hormonal stimulus. Monitoring postprandial plasma levels of GI peptides in snakes with isolated intestinal loops and identifying peptide receptors in such loops could provide evidence of hormones that induce or modulate functional adaptation.

Intestinal response to regulatory peptides. At present, eight python GI peptides have been sequenced (4, 5), and six more peptides have been identified within the python GI tract (22). These peptides and others could be synthesized and infused individually into fasting pythons, to assess their regulatory effects.

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