Metabolic, Ventilatory, and Acid-Base Responses Associated with Specific Dynamic Action in the Toad *Bufo marinus*

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Abstract

Specific dynamic action (SDA), the postprandial increase in metabolic rate that has been well documented in numerous vertebrates, intensifies demand for O_2 delivery by the ventilatory and cardiovascular systems. Yet the well-documented increase in plasma pH following feeding (the "alkaline tide") should, paradoxically, result in a decrease rather than an increase in ventilation. The intent of this study was to investigate in the marine toad Bufo marinus *whether there is a change in the relationship between metabolism, lung ventilation, and blood pH associated specifically with SDA. We measured $\dot{V}O_2$, $\dot{V}CO_2$; respiratory quotient (R_E); lung ventilation volume and frequency; arterial pH, P_{O_2}, and hematocrit; and heart rate before and after the induction of SDA by peptone injection directly into the stomach. Levels of $\dot{V}O_2$ and $\dot{V}CO_2$; approximately 40–60 mL · g^{-1} · h^{-1}, doubled 5–6 h after peptone injection and then declined within 24 h. Accompanying this profound peptone-stimulated SDA, which was dose-dependent, lung ventilation increased from about 3,750 mL · kg^{-1} · h^{-1} to 5,750 mL · kg^{-1} · h^{-1} with a time course similar to that of $\dot{V}O_2$. Heart rate increased from 36 to 45 beats · min^{-1}. In spite of these changes, arterial P_{O_2} and pH (approximately 80 mmHg and 7.75, respectively) did not change during the SDA response. Although the anticipated alkaline tide did not develop in* Bufo, *the large increase in lung ventilation without a corresponding fall in blood pH nonetheless suggests an uncoupling of hyperventilation and an acid stimulus during SDA. Respiratory physiology during the postprandial period clearly deserves more investigation.*

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Introduction

An increase in metabolic rate following food ingestion known as specific dynamic action, or SDA, was initially described in mammals but has since been documented in a wide range of both vertebrates and invertebrates (for references, see Brown and Cameron [1991]; Burggren, Moreira, and Santos [1993]). The basis for the increased metabolism may involve both mechanical and biochemical processing of the meal as well as production of nitrogenous waste products. A growing literature, especially on fish, suggests that protein synthesis may be the most important contributor to SDA (Brown and Cameron 1991; Houlihan 1991; Lyndon, Houlihan, and Hall 1992). That SDA is present in such a diverse array of taxa indicates that the effect of food ingestion, digestion, and subsequent protein synthesis on metabolism is a general component of the metabolic processes of all animals.

Although the metabolic effects of food ingestion and digestion are reasonably well known, the accompanying adjustments in cardiovascular and ventilatory physiology are much less understood. In the toad *Bufo marinus,* both voluntary ingestion of food and bolus injection of peptone increase heart rate (Dumsday 1990) and blood flow to the gut (B. Dumsday and W. Burggren, unpublished data). Increased mesenteric blood flow following feeding also is well described in mammals (cf. Fara 1984) and has been reported in both crocodilian reptiles (Axelson et al. 1991) and fish (Axelson and Fritsche 1991). Attendant on SDA is the so-called alkaline tide, in which plasma pH increases as a result of movements of strong ions (primarily as HCl) into the stomach lumen, which decreases blood chloride levels and causes an increase in blood bicarbonate. This increase in plasma pH can be rather dramatic. For example, in the American alligator, plasma pH can increase as much as 0.4 units within 9 h of feeding (Coulson, Hernandez, and Dessaur 1950).

To date, most studies of SDA and its attendant physiological effects have focused narrowly on particular aspects of metabolic rate and physiological responses. Yet physiological findings from various studies, when viewed together, expose an apparent paradox. On one hand, the postprandial increase in metabolic rate (SDA) intensifies the demand for O_2 delivery by the ventilatory and cardiovascular systems. On the other hand, increased blood pH (and presumably an accompanying increased pH of cerebrospinal fluid, or CSF) following feeding should, according to our conventional understanding of both central and peripheral receptors, decrease ventilation (for reviews, see Milsom [1990]; West and Van Vliet [1992]). Consequently, the paradox lies in how these apparently contradictory physiological responses coexist in the immediate postprandial period.

To understand the interaction between ventilation and metabolism during ingestion and to resolve the outlined paradox, it is imperative to determine metabolic, ventilatory, and hematological responses in one species under the same set of experimental conditions. This study reports on such variables before and after initiation of the SDA response in the marine toad *B. marinus*.

Material and Methods

Experimental Animals

Experiments were carried out on marine toads (*Bufo marinus*) with body weights of 130–450 g. Toads were purchased from a commercial supplier several weeks before experimentation and kept in containers that afforded both dry areas and running water. Animals were fed large crickets and baby mice. Both maintenance and experimental temperatures were 23°–25°C, and light conditions consisted of a daily photoperiod of 12L:12D. Food was withheld for 4–10 d before surgery and/or experimental measurements.

Surgical Preparation for Peptone Injection

These experiments capitalize on the use of peptone injected directly into the stomach as a highly effective method for inducing SDA in amphibians (Dumsday 1990). Peptone (Sigma Chemical) consists of a mixture containing about 65% free amino acids (all common amino acids except tryptophan). Peptone or other materials were delivered via a cannula introduced transabdominally into the stomach, as described in detail by Dumsday (1990). In brief, toads were anesthetized for cannulation with either MS-222 (0.3% triccaine methanesulphonate buffered to pH 7.0) or halothane anesthesia. A flared catheter (PE 90) was connected to a needle (14 gauge) and passed through the esophagus into the stomach and out to the exterior through the overlying body wall. The catheter was secured to the back of the animal with two sutures. All toads tolerated this procedure very well and were allowed to recover for at least 1 d after catheter implantation.

Oxygen Uptake and CO_2 Production

Measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ were carried out on a total of eight toads (130–222 g, mean body mass 185 g) before and after the introduction of peptone and the associated development of a SDA response. These data were determined by flow-through respirometry. The respirometer consisted of a sealed polyvinyl chloride cylinder (6.6 cm in diameter × 26 cm in

length). One end of the cylinder, which contained a gas outlet tube, was permanently sealed. The other end, used for access to the chamber interior, was fitted with a large, tightly fitting rubber stopper containing a gas inlet tube that supplied the interior of the respirometer with humidified air. The stopper also had a small hole through which the stomach catheter exited the respirometer. The hole with the catheter emerging was sealed with modeling clay. The side of the respirometer contained a small glass window for viewing the animal. The interior of the chamber contained a moistened paper towel.

The outlet of the respirometer was connected, in series, to a desiccator tube (containing anhydrous $CaSO_4$), a gas flowmeter, a Beckman OM-11 O_2 analyzer, and a Beckman LB-2 CO_2 analyzer, whose suction pump generated the gas flow through the system (approximately 80 mL · min^{-1}). Both gas analyzers were subjected to a two-point calibration before the start of each experiment. A "shunt" circuit allowed air to be passed very briefly through the analyzers without interrupting gas flow through the respirometers, thus allowing one-point calibrations during the course of the measurements to detect any drift in the analyzer signal.

The decrease in fractional O_2 concentration as gas passed through the respirometer was used to calculate $\dot{V}O_2$ (mL · kg^{-1} · h^{-1}), while the increase in fractional CO_2 concentration was used to calculate $\dot{V}CO_2$ (mL · kg^{-1} · h^{-1}), according to standard equations for calculating these values in flow-through respirometry. The respiratory quotient, R_E, was calculated as $\dot{V}O_2/\dot{V}CO_2$. At the end of each experiment, the $\dot{V}O_2$ and $\dot{V}CO_2$ of the empty chamber were measured to determine the contribution of any microbial $\dot{V}O_2$ and $\dot{V}CO_2$, the values of which were subsequently subtracted.

Surgical Preparation for Blood Sampling

Toads used for measurement of ventilation, blood gases, and blood pressures were additionally chronically cannulated in the right femoral artery as described by Boutilier et al. (1979), under halothane anesthesia. Catheters were filled with heparinized (150 IU/mL) amphibian McKenzie's Ringer and were flushed daily. Toads were allowed to recover from anesthesia for 24–48 h before measurements were initiated.

Blood Analysis

Arterial blood was analyzed for P_{O_2} and pH with Radiometer O_2 and pH electrodes connected to a Radiometer pHM 73 monitor. The O_2 electrode was calibrated daily with borate zero solution (zero values) and immediately

before measurements with humidified atmospheric air (air saturation values). The pH electrode was calibrated with high-precision buffers (S1500 and S1510, Radiometer). Both electrodes were kept at the temperature of the experimental animal. Hemoglobin concentration was determined on an OSM 2 Hemoximeter (Radiometer) in three toads.

Ventilation and Blood Pressure Measurements

Ventilation was measured by the pneumotachographic method described by Glass, Wood, and Johansen (1978), which monitors pressure gradients created by inspiratory and expiratory flows through a flexible rubber mask containing a resistor (Fleisch tube) glued to the head of the toad. All inspired and expired air passed through the resistor. The resulting pressures were monitored by a Statham DP 103–10 differential pressure transducer and recorded with a Sable (V4.1) data acquisition system. Lung ventilation was distinguished from buccal ventilation by the presence of a biphasic flow profile during expiration (Jones 1982). The relationship between the integrated air flow (derived from the pressure signals) and tidal volume was quantified by injection of a range of gas volumes through a catheter implanted in a cast of a toad head fitted to the mask. This produced a very tight correlation ($r^2 = 0.98 \pm 0.01$, $n = 18$), testifying to the high accuracy of the method.

Arterial blood pressure was measured by connecting the implanted catheter to a Narco Bio system pressure transducer (P-1000B) kept at the heart level of the toad. Heart rate was determined by counting pressure pulses. The blood pressures were recorded with a Sable (V4.1) data acquisition system for later analysis.

Experimental Protocol

First, $\dot{V}O_2$ and $\dot{V}CO_2$ were measured in undisturbed toads resting quietly in the respirometers. After a 24–48-h period of recovery from catheter implantation, baseline values of $\dot{V}O_2$ and $\dot{V}CO_2$ were measured during a 2-h period. Then, to determine the effects of saline injection per se, 2.0 mL of 0.9% saline were injected into the gastric catheter, and metabolism over the ensuing 3-h period was measured continuously. Values for $\dot{V}O_2$ and $\dot{V}CO_2$ did not change significantly following a sham injection of saline.

Then, according to a similar protocol, "dose-response" curves for the SDA effect induced by peptone were established. With a new group of toads, measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ were made for each toad before and after injections of saline and then 0.01, 0.1, 1.0, and 10.0 g of peptone. All peptone

doses were suspended in 2.0 mL of 0.9% saline (except for the 10.0-g dose, which had to be suspended in 4 mL to be sufficiently fluid to move through the injection catheter). Each toad was thus measured at least five times (sham plus four doses), with at least 7 d intervening between each experimental run with a new dose of peptone. To determine the time course of the SDA response in greater detail, 1.0-g doses of peptone were injected into a new group of toads. Values for $\dot{V}O_2$ and $\dot{V}CO_2$ were then monitored for the following 24 h.

In the final set of experiments, lung ventilation, heart rate, and blood gases were measured during and after peptone injection. Toads with face masks and arterial cannulae were placed in a environmental chamber on the night before experimentation. On the following morning ventilation and blood pressures were obtained for a 2-h period (serving as control values), after which peptone (1–2 g · 100 g body mass^{-1}) was injected into the stomach. Ventilation and heart rate were monitored continuously and expressed on a hourly basis.

Blood samples were withdrawn immediately before injection of peptone and every second hour thereafter. Blood samples were reinfused after analysis, thus ensuring minimal blood losses.

Although peptone was injected through a catheter passed through an environmental chamber, this injection caused a transient increase in ventilation and heart rate. Similarly, blood sampling caused minor transient increases in these parameters. In order to exclude this effect from the analysis, we omitted in our calculations the data collected during the 15 min following peptone injection and blood sampling.

Statistical Analysis

The effect of peptone injection on the measured parameters was tested for statistical significance with a one-way ANOVA for repeated measures. Differences among means were then assessed with a Student-Newman-Keuls test. A fiducial limit for significance of $P \leq 0.05$ was applied. All data are presented as means ± 1 SEM.

Results

Peptone Effects on Metabolic Rate

Mean resting $\dot{V}O_2$ in fasting toads ranged between 50 and 60 mL O_2 · kg^{-1} · h^{-1} at 23°–25°C. The corresponding $\dot{V}CO_2$ was 40–60 mL CO_2 · kg^{-1} · h^{-1}, producing an R_E of about 0.8.

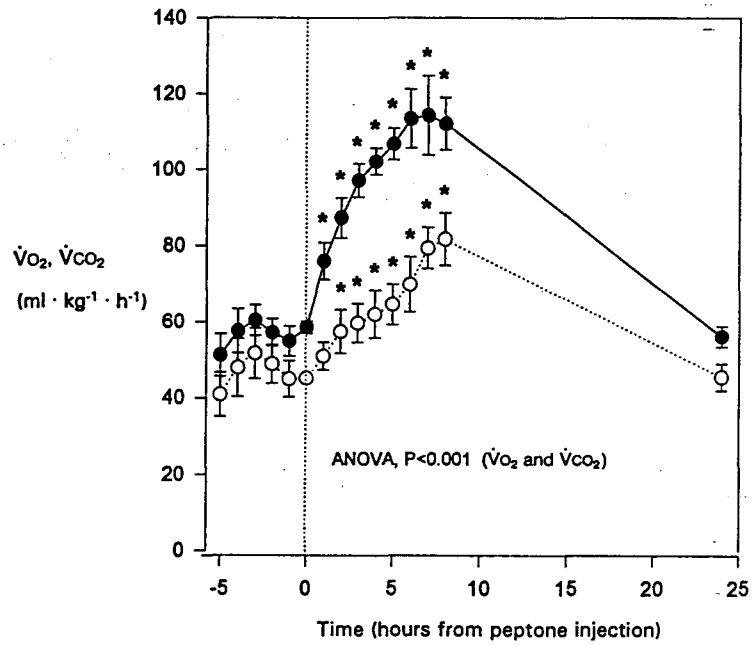

Fig. 1. Levels of $\dot{V}O_2$ (●) and $\dot{V}CO_2$ (○) before and after the injection of 1 g of peptone into the stomach of the toad Bufo marinus. *Data are from four toads; mean body mass is 293 g. Values are* $\bar{X} \pm 1$ *SEM. Results of ANOVA for repeated measures are shown. Asterisks indicate values significantly different* ($P < 0.01$) *from values at time 0.*

Values of $\dot{V}O_2$ and $\dot{V}CO_2$ in fasting *Bufo marinus* before and after the injection of 1 g of peptone into their stomachs are shown in figure 1. Both $\dot{V}O_2$ and $\dot{V}CO_2$ were significantly elevated ($P < 0.001$, $n = 4$) above control (preinjection) values within 2 h. Peak $\dot{V}O_2$ and $\dot{V}CO_2$, about a twofold elevation over preinjection levels, occurred within 5–6 h following peptone injection. The $\dot{V}O_2$ and $\dot{V}CO_2$ had decreased to control levels within 24 h following peptone injection.

The R_E value showed a significant decline ($P < 0.001$, $n = 4$) to about 0.6 within 2 h of peptone injection (fig. 2). The disturbance in R_E followed the same time course as the elevation of $\dot{V}O_2$ and $\dot{V}CO_2$; the R_E returned to values not significantly different from control within 24 h.

The response of $\dot{V}O_2$ to peptone injection was dose-dependent (fig. 3). Peak response to peptone injection occurred with a dose of 1.0 g, which was the dose used for stimulation of SDA in the experiments assessing peptone's affect on metabolism, ventilation, and blood variables.

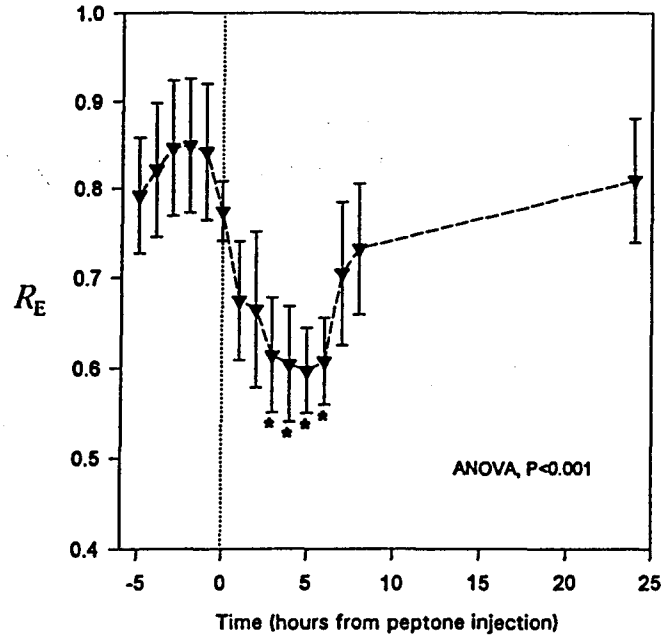

Fig. 2. The R_E *before and after the injection of 1 g of peptone into the stomach of* Bufo marinus. *Values for* R_E *were calculated from the* $\dot{V}O_2$ *and* $\dot{V}CO_2$ *data plotted in fig. 1. Values are* $\bar{X} \pm 1$ *SEM. Results of ANOVA for repeated measures are shown. Asterisks indicate values significantly different* ($P < 0.01$) *from values at time 0.*

Influence of SDA on Ventilation, Heart Rate, and Blood Gases

Ventilation increased significantly ($P = 0.02$, $n = 8$) from 3,750 mL · kg^{-1} · h^{-1} at body temperature and ambient pressure, saturated with water vapor (BTPS), immediately after injection of peptone into the stomach lumen to 5,750 mL about 4 h later. After this rapid increase, ventilation remained stable for the following 8 h at a level approximately 50% above the preinjection level (fig. 4). The increase in ventilation was mirrored by a simultaneous, albeit slower, and highly significant ($P = 0.001$, $n = 6$) increase in heart rate from an average of 36 beats · min^{-1} before injection of peptone to an average of 45 beats · min^{-1} 4–5 h after injection (fig. 5). Heart rate varied considerably among individuals, and the largest relative increases following peptone injection occurred in toads with low initial heart rates.

Arterial P_{O_2} (about 80 mmHg) and pH (about 7.75) remained unchanged following peptone injection (fig. 6). Yet peptone injection caused a significant ($P = 0.006$, $n = 3$) increase in arterial Hb concentration from 9 mM to 12 mM. Arterial Hb levels returned to control level 14 h after peptone injection (data not shown for hour 14).

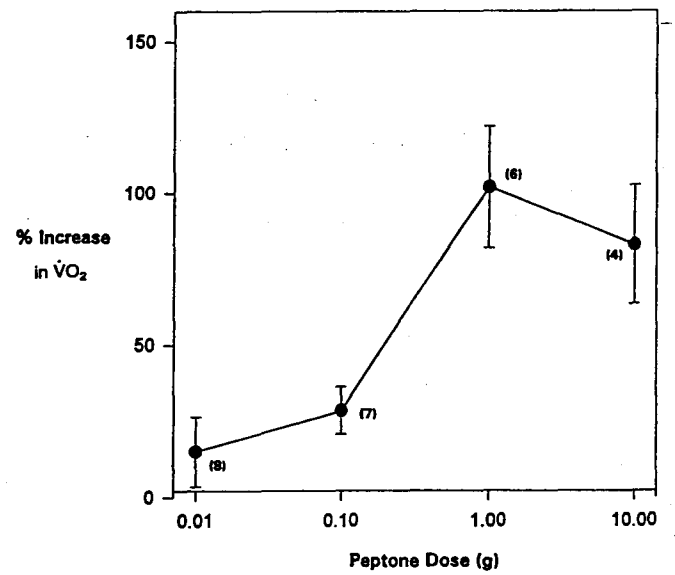

Fig. 3. Dose-response curve of the effect of peptone on $\dot{V}O_2$. Values are the percentage increase over control, measured at hour 6, produced by injection of peptone. Data are from eight toads, mean body mass of 185 g.

Discussion

Comparison of Resting Rates with Values in the Literature

Metabolic rates presented here are similar to those reported earlier for *Bufo marinus* (Shield and Bentley 1973; Feder 1982; Withers et al. 1988; Wood and Malvin 1991). No previous studies provide direct measurements of ventilation in *B. marinus,* and comparison is accordingly restricted to studies on the closely related *Bufo paracnemis,* in which the same methodology has been applied. Our data for resting ventilation are slightly lower than, but consistent with, these studies (Kruhøffer et al. 1987; Branco, Glass, and Hoffmann 1992; Wang, Branco, and Glass 1994). Also, the present data on arterial P_{O_2} and pH are similar to values reported for the unmasked *B. marinus* (Boutilier and Heisler 1988; Wood and Malvin 1991).

In summary, the agreement in physiological values between what we obtained for resting animals and those previously published values strongly suggests that stomach cannulation does not induce severe stress in the toads.

Comparison of SDA in Bufo *and Other Animals*

Specific dynamic action is widely recognized to occur among a wide variety of both vertebrates and invertebrates. The magnitude and time course of

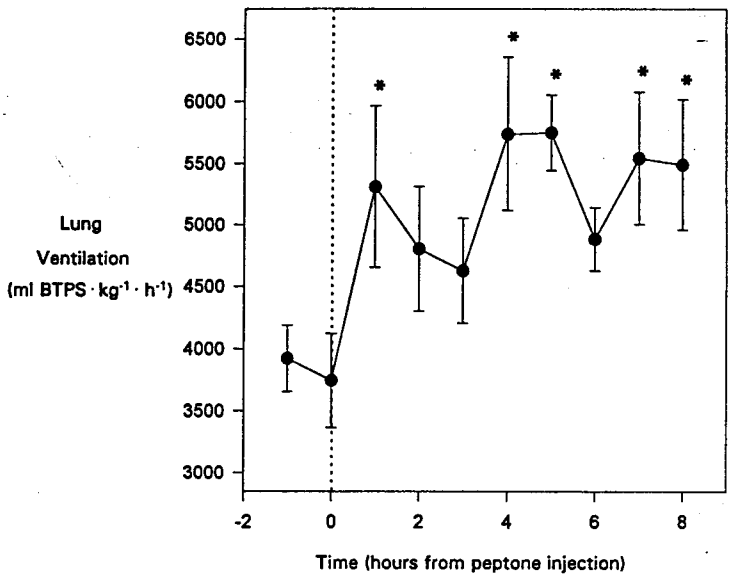

Fig. 4. Lung ventilation before and after the injection of 1–2 g · 100 g^{-1} body weight of peptone into the stomach of Bufo marinus. *Data are from eight toads, mean body mass of 332 g. Values are* $\bar{X} \pm 1$ *SEM. Asterisks indicate values significantly different* ($P < 0.01$) *from values at time 0.*

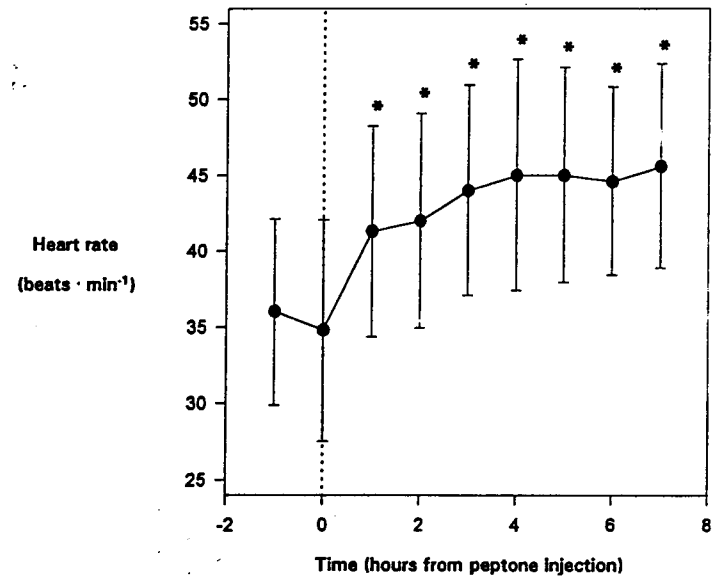

Fig. 5. Heart rate before and after the injection of 1–2 g · 100 g^{-1} body weight of peptone into the stomach of Bufo marinus. *Data are from six toads, mean body mass of 316 g. Values are* $\bar{X} \pm 1$ *SEM. Asterisks indicate values significantly different* ($P < 0.01$) *from values at time 0.*

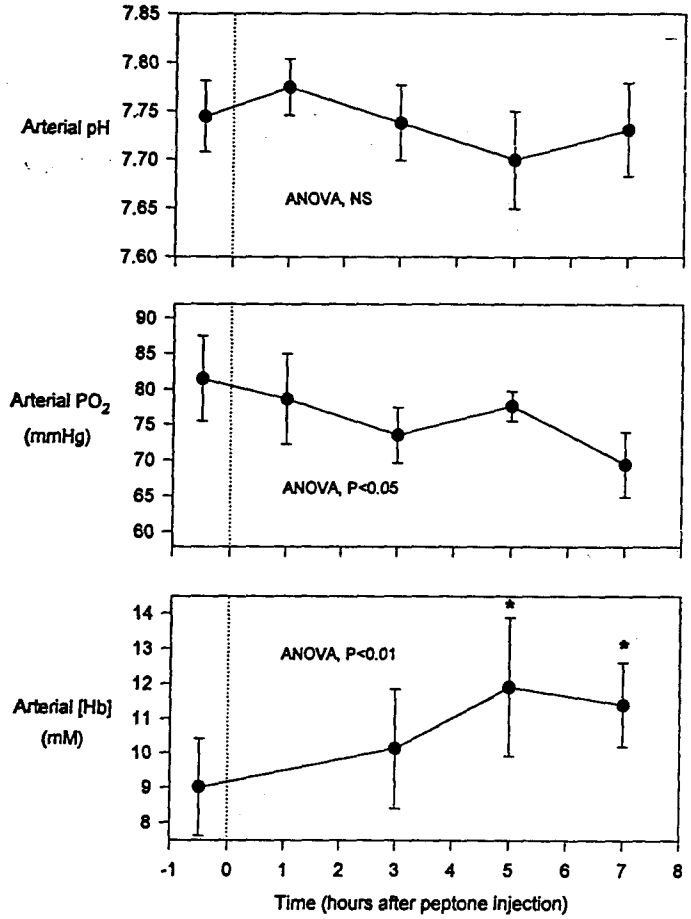

Fig. 6. Arterial blood P_{O_2}, pH, and Hb concentration before and after the injection of 1–2 g · 100 g^{-1} body weight of peptone into the stomach of Bufo marinus. *For P_{O_2} and pH, data are from six toads, mean body mass of 302 g; for Hb concentration, data are from three toads, mean body mass of 364 g. Values are* $\bar{X} \pm 1$ *SEM. Results of ANOVA for repeated measures are shown. Asterisks indicate values significantly different (*$P < 0.01$*) from values at time 0.*

the SDA response is dependent on the species, its feeding habits, and the manner used to stimulate SDA. In general, intermittent feeders, such as snakes and alligators, with days or weeks between feedings, show larger increases in metabolism than do frequent feeders. For example, boid snakes increase $\dot{V}_{O_2}$ almost 10 times after feeding (T. Wang and A. S. Abe, unpublished data), whereas the $\dot{V}_{O_2}$ of frequently feeding animals (many fishes and omnivorous crabs) may increase by only 20%–100%. This difference

may be explained at least partly by the lower resting metabolism in intermittent feeders rather than by an exaggerated SDA response.

The SDA response in *B. marinus,* an increase in O_2 consumption of about 100% within 6 h (fig. 1), agrees well with that induced experimentally through amino acid infusion or force-feeding in animals such as trout (Brown and Cameron 1991) or littoral air-breathing crabs (Burggren, Moreira, and Santos 1993). The magnitude of the O_2 consumption increase also approximately parallels the heart rate response in *B. marinus* induced by gastric injection of peptone (fig. 5; see also Dumsday 1990). However, the maximum heart rate induced by peptone is far more rapid, reaching peak values by 1 h rather than the 4–6 h observed in the present study for $\dot{V}O_2$. Whether the relationship between $\dot{V}O_2$ and heart rate is causal or correlative—that is, whether the increase in heart rate and attendant increase in blood flow supports the elevated $\dot{V}O_2$—remains unclear but would be an interesting avenue for further research.

Blood Gas Transport and Acid-Base Balance during SDA

Arterial P_{O_2} and pH did not change during the SDA, but there was a 25% increase in Hb concentration. It is clear that, in combination with the increased heart rate (and presumed increase in cardiac output), blood O_2 delivery to peripheral tissues increased concomitantly with the SDA-induced rise in aerobic metabolic rate.

Unlike in alligators and man, there was no alkaline tide observed in toads. Arterial acid-base status may nevertheless have changed as a function of feeding. Formation of gastric acid involves an intricate set of ion exchanges in parietal cells (Stevens 1988), resulting in a net increase in plasma $[HCO_3^-]$ and an acidification of the stomach lumen. The decrease in R_E (fig. 2) and resulting CO_2 retention after feeding is entirely consistent with such an increase in plasma HCO_3^- levels. It is therefore likely that the unaffected arterial pH results from a simultaneous increase in arterial P_{CO_2} caused by a decrease in ventilation relative to CO_2 production. In other words, a respiratory compensation to a metabolic alkalosis may have taken place. Although we did not determine the partitioning of gas exchange, this possibility is supported by the fact that the increase in ventilation following peptone injection was lower than the observed increase in CO_2 elimination (figs. 1, 4).

The SDA Paradox

In air-breathing vertebrates, ventilation is primarily controlled to maintain constant arterial blood gases, and a number of studies document alterations

in arterial pH as a powerful and dominant stimulus (Milsom 1990). Although the anticipated alkaline tide accompanying SDA did not develop in *B. marinus* (i.e., plasma pH was unchanged), there was nonetheless a 50% increase in lung ventilation. Our experiments after peptone injections demonstrated that the relationship between plasma (and presumably CSF) pH and lung ventilation typically investigated in fasting vertebrates (cf. Milsom 1990; West and Van Vliet 1992) cannot adequately explain the relationship between ventilation and body-fluid pH in the postprandial period. A specific, pH-independent CO_2 stimulus on the central chemoreceptor has been demonstrated in mammals (Shams 1985). If present in *Bufo* as well, at least part of the increased ventilation following peptone injection could be attributed to increased arterial P_{CO_2}. Feeding and the associated SDA are an important component of any animal's life history, and the physiological responses and changes associated with this non-steady-state condition deserve far more attention than they have received to date.

Acknowledgments

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Literature Cited

Axelson, M., and R. Fritsche. 1991. Effects of exercise, hypoxia and feeding on the gastrointestinal blood flow in the Atlantic cod *Gadus morhua*. J. Exp. Biol. 158: 181–198.

Axelson, M., R. Fritsche, S. Holmgren, D. Grove, and S. Nilsson. 1991. Effects of diving, feeding and drugs on the gastrointestinal blood flow in the estuarine crocodile, *Crocodylus porosus*. Acta Physiol. Scand. 142:509–516.

Boutilier, R. G., and N. Heisler. 1988. Acid-base regulation and blood gases in the anuran amphibian, *Bufo marinus*, during environmental hypercapnia. J. Exp. Biol. 134:79–98.

Boutilier, R. G., D. J. Randall, G. Shelton, and D. P. Toews. 1979. Acid-base relationships in the blood of the toad *Bufo marinus*. I. The effects of environmental CO_2. J. Exp. Biol. 82:331–344.

Branco, L. G. S., M. L. Glass, and A. Hoffmann. 1992. Central chemoreceptor drive to breathing in unanesthetized toads, *Bufo paracmenis*. Respir. Physiol. 87:195–207.

Brown, C. R., and J. N. Cameron. 1991. The induction of specific dynamic action in channel catfish by infusion of essential amino acids. Physiol. Zool. 64:276–297.

BURGGREN, W. W., G. S. MOREIRA, and M. C. F. SANTOS. 1993. Specific dynamic action and the metabolism of the brachyuran land crabs *Ocypode quadrata, Goniopsis cruentata* and *Cardiosoma guanhumi.* J. Exp. Mar. Biol. Ecol. 169:117–130.

COULSON, R. A., T. HERNANDEZ, and H. C. DESSAUR. 1950. Alkaline tide of the alligator. Proc. Soc. Exp. Biol. Med. 74:866–869.

DUMSDAY, B. 1990. Resting heart rate of the toad *Bufo marinus:* a long-term study of individual differences and environmental influences. Physiol. Zool. 63:420–431.

FARA, J. W. 1984. Postprandial mesenteric hyperemia. Pages 99–106 in A. P. SHEPHARD and D. N. GRANGER, eds. Physiology of the intestinal circulation. Raven, New York.

FEDER, M. E. 1982. Environmental variability and thermal acclimation of metabolism in tropical anurans. J. Therm. Biol. 7:23–28.

GLASS, M. L., S. C. WOOD, and K. JOHANSEN. 1978. The application of pneumotachography on small unrestrained animals. Comp. Biochem. Physiol. 59A:425–427.

HOULIHAN, D. F. 1991. Protein turnover in ectotherms and its relationships to energetics. Pages 1–43 in R. GILLES, ed. Advances in comparative and environmental physiology. Vol. 7. Springer, Berlin.

JONES, R. M. 1982. How toads breathe: control of air flow to and from the lung by the nares in *Bufo marinus.* Respir. Physiol. 49:251–265.

KRUHØFFER, M., M. L. GLASS, A. S. ABE, and K. JOHANSEN. 1987. Control of breathing in an amphibian *Bufo paracnemis:* effects of temperature and hypoxia. Respir. Physiol. 69:267–275.

LYNDON, A. R., D. F. HOULIHAN, and S. J. HALL. 1992. The effect of short term fasting and a single meal on protein synthesis and oxygen consumption in cod, *Gadus morbua.* J. Comp. Physiol. 162B:209–215.

MILSOM, W. K. 1990. Control and co-ordination of gas exchange in air breathers. Pages 347–400 in R. G. BOUTILIER, ed. Advances in comparative and environmental physiology. Vol. 6. Springer, Berlin.

SHAMS, H. 1985. Differential effects of CO_2 and H^+ as central stimuli of respiration in the cat. J. Appl. Physiol. 58:357–364.

SHIELD, J. W., and P. J. BENTLEY. 1973. Respiration of some urodele and anuran Amphibia. I. In water, role of the skin and gills. Comp. Biochem. Physiol. 46A:17–28.

STEVENS, C. E. 1988. Comparative physiology of the vertebrate digestive system. Cambridge University Press, Cambridge.

WANG, T., L. G. S. BRANCO, and M. L. GLASS. 1994. Ventilatory responses to hypoxia in the toad *Bufo paracnemis* before and after a decrease in hemoglobin oxygen-carrying capacity. J. Exp. Biol. 186:1–8.

WEST, N. H., and B. N. VAN VLIET. 1992. Sensory mechanisms regulating the cardiovascular and respiratory systems. Pages 151–182 in M. E. FEDER and W. W. BURGGREN, eds. Environmental physiology of the amphibians. University of Chicago Press, Chicago.

WITHERS, P. C., S. S. HILLMAN, L. A. SIMMONS, and A. C. ZYGMUNT. 1988. Cardiovascular adjustments to enforced activity in the anuran amphibian, *Bufo marinus.* Comp. Biochem. Physiol. 89A:45–49.

WOOD, S. C., and G. M. MALVIN. 1991. Physiological significance of behavioral hypothermia in hypoxic toads (*Bufo marinus*). J. Exp. Biol. 159:203–215.