Effects of feeding on metabolism, gas transport, and acid-base balance in the bullfrog Rana catesbeiana

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1Centre for Respiratory Adaptation, Institute of Biology, University of Southern Denmark, Main Campus: Odense University, DK-5230 Odense M, Denmark; and 2School of Biological Sciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom

Busk, Morten, Frank B. Jensen, and Tobias Wang. Effects of feeding on metabolism, gas transport, and acid-base balance in the bullfrog Rana catesbeiana. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R185–R195, 2000.—Massive feeding in ectothermic vertebrates causes changes in metabolism and acid-base and respiratory parameters. Most investigations have focused on only one aspect of these complex changes, and different species have been used, making comparison among studies difficult. The purpose of the present study was, therefore, to provide an integrative study of the multiple physiological changes taking place after feeding. Bullfrogs (Rana catesbeiana) partly submerged in water were fed meals (mice or rats) amounting to ~0.2% of their body weight. Oxygen consumption increased and peaked at a value three times the predigestive level 72–96 h after feeding. Arterial Po2 decreased slightly during digestion, whereas hemoglobin-bound oxygen saturation was unaffected. Yet, arterial blood oxygen content was pronouncedly elevated because of a 60% increase in hematocrit, which appeared mediated via release of red blood cells from the spleen. Gastric acid secretion was associated with a 60% increase in plasma HCO3 concentration ([HCO3]) 48 h after feeding. Arterial pH only increased from 7.86 to 7.94, because the metabolic alkalosis was countered by an increase in Pco2 from 10.8 to 13.7 mmHg. Feeding also induced a small intracellular alkalosis in the sartorius muscle. Arterial pH and HCO3 returned to control values 96–120 h after feeding. There was no sign of anaerobic energy production during digestion as plasma and tissue lactate levels remained low and intracellular ATP concentration stayed high. However, phosphocreatine was reduced in the sartorius muscle and ventricle 48 h after feeding.

specific dynamic action; O2 transport; alkaline tide; metabolites; high-energy phosphates

ECTOTHERMIC CARNIVORES VERTEBRATES habitually ingest very large meals that induce several-fold increases in oxygen uptake (V˙O2). This response, often referred to as specific dynamic action (SDA), varies among species and depends on the size of the meal. In snakes, V˙O2 as specific dynamic action (SDA), varies among species and depends on the size of the meal. In snakes, V˙O2 increases more than 10-fold after feeding (1, 28), and the peak value exceeds that attained during muscular exercise (1, 27). Endothermic vertebrates, in contrast, have high standard metabolic rates and typically feed often and on relatively small meals, limiting the magnitude of their SDA. Many physiological and biochemical factors contribute to SDA. Preabsorptive factors include mechanical processing of the meal, secretion of digestive fluids into the gastrointestinal tract, upregulation of digestive enzyme and nutrient transporter levels, and, in some cases, extensive intestinal hypertrophy (17, 30). Postabsorptive factors include transmembrane transfer of nutrient into target organs and protein synthesis.

The elevated metabolism after feeding places increased demands on the energy transport by the cardiopulmonary system, but respiratory physiology during digestion remains little studied. In toads, SDA has been elicited by peptone injection into the stomach (36), which leads to an increased heart rate (10) and an elevation of blood hemoglobin concentration, whereas arterial Po2 (Pao2) remains high. In contrast, it has been reported that Pao2 decreases drastically during digestion in pythons. Feeding has also been reported to increase plasma [HCO3] and pH (“alkaline tide”) as a result of acid secretion into the stomach in reptiles (8, 27). However, in these studies, blood was sampled by cardiac puncture from conscious animals, which is likely to affect blood pH due to struggling proceeding sampling. Furthermore, as cardiac puncture yields an uncertain mixture of venous and arterial blood, the reported P02 and Pco2 measurements are unlikely to reflect arterial blood gases of undisturbed animals. Thus, at present, there are very limited data on blood gases and acid-base parameters in undisturbed animals after feeding, and, in some cases, the findings are conflicting. A major goal of the present study was, therefore, to determine acid-base status and respiratory parameters of arterial blood in the frog Rana catesbeiana after feeding. Because of the intracellular presence of pH-sensitive enzymes, intracellular acid-base status may be regulated at the expense of the extracellular space. However, there are no reports on intracellular acid-base status during digestion. Accordingly, to provide an integrated picture of whole animal acid-base regulation, intracellular acid-base status was also determined.

A significant contribution from anaerobic metabolism to energy production during digestion is unlikely, considering the intensity and duration (several days) of the SDA response and the low efficiency of anaerobic pathways. This does not, however, exclude a significant effect of anaerobic metabolism on acid-base status in...
the extracellular space or in selected organs. Peripheral tissues (e.g., skeletal muscles) and central aerobic organs (e.g., liver, kidney, and ventricle) could experience oxygen delivery limitations because of tissue-dependent changes in blood flow and/or oxygen demand during digestion. A final goal was, therefore, to investigate key metabolites (lactate and high-energy phosphates) in selected body compartments to evaluate metabolism in the postprandial period.

MATERIALS AND METHODS

Experimental Animals

Adult bullfrogs (Rana catesbeiana) weighing 358–588 g (mean ± SE: 421 ± 14 g, n = 20) were obtained from North Carolina Biological Supply or Charles D. Sullivan several months before experimentation and kept in large plastic cages with free access to tap water at 20 ± 2°C. A light bulb provided a basking site for thermoregulation and a 12:12-h light-dark rhythm. Frogs were force-fed liver or heart (~10% of body wt) once a week, but starved for 2 wk before experimentation to ensure that digestion was completed. For experimentation, frogs were fed mice or rats equivalent to 10% of their body weight (see Experimental Protocol). Most reports on massive feeding in anurans are anecdotal, but several sit-and-wait foraging species, including Rana catesbeiana, often feed on large prey items such as mammals, reptiles, and other anurans (9). Accordingly, a meal size amounting to 10% of the body mass seems realistic. To our knowledge, there are no data for clearance rates of food after an oral meal of body weight (see Table). To our knowledge, there are no data for clearance rates of food after an oral meal of body weight.

Experimental Protocol

Series I: Oxygen consumption and carbon dioxide excretion. O₂ consumption and carbon dioxide production (VCO₂) were determined on uncatheterized frogs (445 ± 44 g, n = 5) with closed-system respirometry using 2.6-liter chambers supplied with flowing water (covering the bottom) and air during nonmeasurement periods. The whole system was thermostatted at 22 ± 1°C. Frogs were placed in the chambers and allowed to acclimate for ~24 h before measurements were started. During measurements, the chambers were sealed for 20–60 min. Air samples were drawn at the beginning and the end of this period and analyzed for O₂ and CO₂, with a Datex (Helsinki, Finland) Normocap 200 gas analyzer. Gas exchange between animal and the small water volume (~100 ml) remaining in the sealed chamber was considered insignificant. After measurement of standard metabolic rate (VO₂ and VCO₂), frogs were fed mice or rats (44 ± 4 g). The frogs did not eat voluntarily but did swallow the meal whenever it was placed in the mouth. Measurements were then repeated with 24-h intervals until 144 h after feeding. The respiratory gas exchange ratio was calculated as VCO₂/VO₂.

Series II: Blood and transepithelial flux measurements. For experiments involving blood and tissue sampling, frogs were anesthetized by immersion in a 1-g/l solution of MS-222 (3-aminobenzoic acid ethyl ester; Sigma) and subsequently cannulated in the right femoral artery (4). Catheters were filled with heparinized saline and flushed daily. After 24 h of recovery from anesthesia, catheterized frogs (424 ± 10 g, n = 8) were transferred to an experimental chamber with a volume of 16 liters. The chamber was fitted with a transparent lid, but the sides were covered with black plastic to minimize visual disturbance. The bottom was covered with 2.5 liters of aerated water at 22 ± 1°C. Tetraacyclidine hydroxide (12.5 mg/l) was added to the water to prevent any bacterial growth. To determine transepithelial fluxes (skin and/or renal system) of acid-base equivalents and blood acid-base and respiratory parameters, blood and water samples were taken according to the following protocol. Frogs were allowed a 24-h acclimation period in the experimental chamber, whereupon the first water sample (beginning of the control flux period) was taken. After 24 h, a second water sample was taken. A 1-ml control blood sample was subsequently drawn (defined as time 0) and analyzed for acid-base and respiratory parameters. Remaining blood was centrifuged (3,000 rpm for 2 min) to separate plasma and red blood cells (RBCs). The plasma was stored at −80°C, and the RBCs were resuspended in amphibian Ringer and reinfused into the animals. Immediately after the control sampling of blood, each frog was fed freshly killed mice or rats (37 ± 2 g). When the meal was swallowed, 1.5 liters of the water in the chamber was changed and shortly thereafter a water sample was taken. The procedure for blood/water sampling and water shift was repeated every 24 h until 144 h after feeding. Occasionally, experiments had to be terminated before 144 h due to clotting of the catheter. To evaluate if blood chemistry was influenced by repetitive blood sampling per se, a control group of frogs (412 ± 11 g, n = 3) was treated as described above, except that the frogs were not fed.

Analytic Procedures

Blood measurements. Arterial blood was analyzed for PaO₂, and pH using a Radiometer (Copenhagen, Denmark) O₂ electrode (E5046–0) and a capillary pH electrode (PS-1 204), respectively. Both electrodes were maintained at 22°C in a BMS Mk3 electrode assembly. Total contents of O₂ (CO₂) and CO₂ (CCO₂) in whole blood and plasma were measured as described by Tucker (34) and Cameron (6) and corrected according to Bridges et al. (5). Arterial PCO₂ (PACO₂) was calculated from the Henderson-Hasselbalch equation using pH and PCO₂ solubility values (γCO₂) from Heisler (13). The plasma HCO₃⁻ concentration ([HCO₃⁻]) was calculated as plasma CCO₂ − αCO₂ × PCO₂. Hematocrit (Hct) was determined after 3 min centrifugation at 12,000 rpm in capillary tubes. The apparent RBC bicarbonate concentration ([HCO₃⁻]RBC) was calculated from the plasma ([HCO₃⁻] and whole blood (wb)
CCO₂ and Hct using the formula: 
\[ [\text{HCO}_3^-]_{\text{RBC}} = \frac{[\text{CCO}_2]_{\text{RBC}} - [\text{Hct}/100][\text{CCO}_2]_{\text{pl}}(0.01\times \text{Hct}) - P_{\text{CO}_2} \times \alpha_{\text{CO}_2} \times [\text{HCO}_3^-]_{\text{RBC}}}{1 - (\text{Hct}/100)} \times \alpha_{\text{CO}_2} \times [\text{HCO}_3^-]_{\text{RBC}} \]
was expressed per kilogram of RBC water, assuming a fractional water content of 0.71 as determined for Bufo marinus (19). Hemoglobin-bound oxygen (HbO₂) was calculated as HbO₂ relative to the O₂ capacity of the functional Hb (i.e., total Hb - Hbmet). Plasma chloride was measured by coulometric titration (Radiometer CMT 10), and sodium and potassium were measured by flame photometry (Instrumentation Laboratory 243). Plasma osmolality was measured by a cryoscopic osmometer (Gonotec Osmomat 030), and plasma protein was measured with the Lowry method (18).

Intracellular acid-base parameters and metabolite measurements. Intracellular pH and total CO₂ in the sartorius muscle were determined in tissue homogenates as described by Pörtner et al. (23). In contrast, frozen tissue samples were placed under liquid nitrogen and metabolism was arrested using 130 mM potassium fluoride (KF) and 5 mM nitritetriacetic acid (NTA). pH and total CO₂ of the supernatant were measured as described above. Total CO₂ was corrected for contamination with extracellular CO₂ (23). Intracellular water content and extracellular water content of sartorius muscle were approximated by values for the triceps femoris and gracilis complex (38). Finally, intracellular [HCO₃⁻] was calculated as described for plasma, using pK₃ and CO₂ solubility values from Heisler (13). Intracellular metabolites (lactate, total ammonium, creatine phosphate, creatine, and ATP) were analyzed in sartorius, ventricle, and liver by standard enzymatic tests after tissue extraction in 8% HClO₄ and neutralization with 3 M K₂CO₃ and 0.5 M HOCH₂CH₃⁻.

Intracellular nonbicarbonate buffer values. The nonbicarbonate buffer value for sarcolemma was calculated as described by Pörtner (22). Approximately 1 g of tissue powder was added to four times the tissue volume of ice-cold metabolic inhibitor solution (in mM: 540 KF, 10 NTA, and 5 KHC₃O₃) and briefly vortexed. The tissue homogenate was equilibrated with 0.5, 3, and 7% humidified CO₂ (delivered by cascaded Wösthoff gas mixing pumps) in Eschweiler (Kiel, Germany) tonometers, and the supernatant was analyzed for pH and total CO₂ as described above. α₉CO₂ was calculated according to Heisler (13), using an osmolality of 850 mM (osmolality of the supernatant).

Transmural transfer of acid-base equivalents. Water samples (10 ml) were equilibrated to 1% humidified CO₂ in a gas-tight titration beaker thermostated at 30°C. To determine changes in strong ion difference (Δ(S/ID)), water samples collected at the end of each flux period were titrated back to the pH of the water samples collected at the beginning of each flux period by adding either HCl or NaHCO₃ from 120 mM standards. pH was measured with a Radiometer combined pH electrode (GK2401C). Finally, water samples were analyzed for total ammonia ([NH₃] + [NH₄⁺]), with the phenylhypochlorite method (32) to determine the total transfer of H⁺ equivalents [Δ([NH₃] + [NH₄⁺]) - Δ(S/ID)] between animal and environmental water. The total transfer of H⁺ equivalents was expressed as a flux rate considering the water volume of the chamber and the body mass of the frogs.

Statistical Analysis

For the metabolic rate, blood, and transepithelial flux measurements, statistical differences were tested by one-way ANOVA for repeated measurements followed by a Student-Newman-Keuls test. For the intracellular measurements, an unpaired t-test or (if the normality and/or equal variance test failed) the Mann-Whitney rank sum test was used. Differences were accepted to be significant at P < 0.05.

RESULTS

\[ \text{V}_\text{O}_2 \text{ and } \text{V}_\text{CO}_2 \]

The \text{V}_\text{O}_2 of fasting animals at rest was 27 ml \text{O}_2·kg⁻¹·h⁻¹ (Fig. 1A), which is equivalent to a standard metabolic rate of 0.54 kJ·kg⁻¹·h⁻¹. After feeding, \text{V}_\text{O}_2 increased significantly, peaking at a value approximately three times higher than the preigestive value. After feeding, \text{V}_\text{CO}_2 remained significantly elevated above the time zero fasting value throughout the experimental period (Fig. 1A). \text{V}_\text{CO}_2 more or less paralleled \text{V}_\text{O}_2 (Fig. 1A), whereby feeding only induced

![Fig. 1. Changes in oxygen uptake (\text{V}_\text{O}_2, \circ) and carbon dioxide release (\text{V}_\text{CO}_2, \text{□}) and in respiratory gas exchange ratio (R_E; B) in Rana catesbeiana after a food intake of −\text{1}⁄{10} of their body weight. Values at time 0 are control values obtained just before feeding. Means ± SE; n = 5. *Significant difference from control values.]
a minor and nonsignificant decrease in the respiratory gas exchange ratio (Fig. 1B).

Blood Respiratory Parameters

$P_{O_2}$ displayed a minor and nonsignificant decrease after feeding (Fig. 2A), and $HbO_2$-sat (Fig. 2B) remained virtually unaffected. Arterial $O_2$ content, however, was significantly increased throughout the experimental period (Fig. 2C). This was due to a significant increase in Hct (Fig. 3A) and hemoglobin concentration (Fig. 3B) with the mean cellular hemoglobin concentration staying constant (Fig. 3C). There were no changes in blood respiratory parameters with time in unfed control animals (Table 1).

Fig. 2. Changes in arterial oxygen tension ($P_{O_2}$; A), oxygen saturation ($HbO_2$; B), and oxygen content (C) in Rana catesbeiana after food intake of $\frac{1}{10}$ of body weight. Means $\pm$ SE; n = 8, 8, 8, 7, 7, 4, and 3 at times 0 (just before feeding), 24, 48, 72, 96, 120, and 144 h after feeding, respectively. *Significant difference from time 0 predigestive values.

Fig. 3. Changes in arterial blood hematocrit (A), total hemoglobin content (B), and mean cellular hemoglobin content (MCHC; C) after feeding. Other details as in Fig. 2. *Significant difference from time 0 predigestive values.

Extra- and Intracellular Acid-Base Parameters and Nonbicarbonate Buffer Values

Feeding resulted in a pronounced increase in the plasma bicarbonate concentration from a control value of 24.2 to a maximum value of 37.8 mM at 48 h (Fig. 4B). Feeding also elevated $P_{CO_2}$ from 10.8 to 13.7 mmHg within 48 h, where after it remained nearly constant throughout the experiment (Fig. 4C). Thus the feeding-induced metabolic alkalosis was partially compensated by a concomitant respiratory acidosis, resulting in an only minor rise in pH of 0.08–0.09 pH units at 24–48 h (Fig. 4A). The pH compensatory effect of the respiratory acidosis is best illustrated in a Davenport diagram, where the pH values expected if only the respiratory acidosis were present, is included for comparison (Fig. 5). At 96 h, pH was back to the control
value, but then decreased to a significantly lower value 144 h after feeding (Fig. 4A). The postprandial increase in plasma [HCO₃⁻] was paralleled by an increase in RBC [HCO₃⁻] (Fig. 4B), but changes in the latter were nonsignificant. Repetitive blood sampling per se did not cause time-dependent changes in arterial acid-base parameters (Table 1).

Feeding also resulted in a small intracellular alkalosis (ΔpH = 0.05) in the sartorius muscle 48 h after feeding (Table 2). The intracellular nonbicarbonate buffer value of sartorius muscle was not significantly affected by feeding. It was 40.6 ± 6.1 mmol·P/H unit⁻¹·kg⁻¹ tissue (n = 3) before feeding and 34.5 ± 1.4 mmol·P/H unit⁻¹·kg⁻¹ tissue (n = 3) after feeding.

Transepithelial Fluxes of Acid-Base Equivalents and Ammonia

The total ammonia excretion rate increased strongly from a low value of 9 µmol·kg⁻¹·h⁻¹ before feeding to ~400 µmol·kg⁻¹·h⁻¹ between 72 and 120 h after feeding (Fig. 6A). The predigestive H⁺ excretion rate was low and not significantly different from zero, and the H⁺ excretion rates after feeding were not significantly different from the control value (Fig. 6B).

Plasma Osmolality, Protein Concentration, and Ionic Status

Plasma osmolality (Fig. 7A) and plasma protein concentration (Fig. 7B) did not change significantly after feeding. Plasma [Na⁺] and [K⁺] did not change (Fig. 8, A and B, respectively), whereas plasma [Cl⁻] declined significantly (Fig. 8C) in the postprandial period. Changes in plasma [Cl⁻] and [HCO₃⁻] were close to showing an inverse 1:1 molar relationship (Fig. 9). Blood sampling per se had no effect on plasma osmolality, protein concentration, or ionic status (Table 1).

Extra- and Intracellular Metabolite Status

The plasma total ammonia content was significantly increased 96 h after feeding (Fig. 10A), but lactate remained low and nearly constant during the whole postprandial period (Fig. 10B). There were no significant changes in these parameters in nonfed control animals (Table 1). The extracellular increase in the ammonia concentration was accompanied by a significant increase in the sartorius muscle ammonia concentration 48 h after feeding (Table 3). Feeding resulted in significant decreases in the phosphocreatine content of sartorius muscle and ventricle (Table 3). The postprandial drop in ventricular phosphocreatine level was more or less mirrored by a concomitant, although nonsignificant, increase in the creatine content, whereas the sartorius muscle creatine content stayed constant. Despite the drop in phosphocreatine levels, ATP remained constant in all tissues after feeding (Table 3).

DISCUSSION

VCO₂

The 200% postprandial increase in metabolic rate (SDA) observed in this study (Fig. 1A) was higher than the maximal 100% increase observed in Bufo marinus after injections of various amounts of amino acids into the stomach (36), but within the range that has been reported in most species of reptiles after feeding (30–320%; Ref. 35). The most dramatic feeding-induced increases in metabolic rate (up to 4,400%) have been reported in sit-and-wait foraging snakes (28). The peak VO₂ in the bullfrogs was reached 96 h after feeding (Fig. 1), compared with <12 h in fish (16), ~24 h in a monitor lizard (29), and up to 48 h in snakes (12, 28, 30). The heterogeneous values reported for intensity and duration of SDA is probably due to differences in meal size and composition, temperature, extent of hypertrophy of the gut, and standard metabolic rate.

The predigestive respiratory gas exchange ratio of ~0.8 (Fig. 1B) suggests oxidation of a mixture of lipid, protein, and carbohydrate substrates. Although nonsignificant, the decrease in the respiratory ratio to ~0.7 throughout the postprandial period may be related to a
shift to a nearly exclusively lipid-based ATP production. The increased ammonium excretion (Fig. 6A), however, suggests increased oxidation of amino acids as well.

**Blood Respiratory Parameters**

$P_{a_{O2}}$ and $HbO_2$-sat remained high during digestion (Fig. 2A and B) as previously reported for chronically cannulated Bufo marinus after peptone injection into the stomach (36). Because of central vascular right-to-left shunt, systemic arterial blood in anurans constitutes a mixture of systemic venous blood and pulmonary venous blood (37). Right-to-left shunt decreases systemic arterial $O_2$ levels relative to left atrial blood and most likely explains why $HbO_2$-sat was ~0.9 (Fig. 2B). The constant $HbO_2$-sat throughout the experiment may indicate that the right-to-left shunt was not affected by digestion, but a complete analysis would have required obtaining additional blood samples from the left and right atria. Nevertheless, it can be concluded that blood in the pulmonary circulation attains virtually full $HbO_2$-sat during the postprandial period. In contrast to our study and the study of Wang et al. (36), Secor and Diamond (27) reported that blood $P_{O2}$ of pythons plunges to ~20 mmHg during digestion. However, because blood samples were obtained by cardiac puncture, this value is unlikely to reflect arterial values in undisturbed animals.

The increased Hct (Fig. 3A) and Hb concentration (Fig. 3B) after feeding is in line with the finding of Wang et al. (36) that hemoglobin concentration increases after injection of amino acids into the stomach of the toad Bufo marinus. To our knowledge, there are no other reports on changes in hematological parameters in lower vertebrates after feeding. The increase in Hct may result from release of RBCs from the spleen or a reduction in plasma volume caused by water shift to intracellular body compartments. Furthermore, a water shift to the gastrointestinal system, driven by secretion of osmotic active solutes (e.g., chloride) or disarticulation of the prey, is possible. Considering the increased demand for oxygen transport by the cardiovascular system during digestion, a release of RBCs seems likely. The increase in Hct observed in many fish species during hypoxia results, at least partly, from splenic contractions (14). However, the increase in Hct caused by hypoxic exposure in cold-submerged Rana catesbeiana was due to decreased plasma volume rather than release of RBCs from the spleen (20). There was no indication of RBC swelling in the present study, as the mean cellular Hb content stayed constant throughout the experiment (Fig. 3C). Furthermore, there were only small changes in plasma osmolality (Fig. 7A) and protein (Fig. 7B), and intracellular lactate remained low (Table 3). Thus, a reduction of the plasma volume caused by osmotic water movement to the intracellular space or stomach seems unlikely. It is suggested that the increase in Hct and Hb concentration is due to a release of RBCs from the spleen to improve oxygen transport. In support of this, changes in Hct and Hb concentration and changes in metabolic rate showed similar time courses (Figs. 1A and 3A and B). Furthermore, the frogs with the lowest Hct values before feeding attained the highest Hct values after feeding (Fig. 11), which seems difficult to relate to osmotic fluid shifts. One reason for this correlation could be that a low predigestive Hct value correlates with depressed metabolic activities (including digestive activities). Thus a higher postprandial $V_O2$ is needed to prepare those animals for digestion.

**Intra- and Extracellular Acid-Base Status**

Repetitive blood sampling per se had no effect on blood acid-base parameters (Table 1). However, it must be noted that the arterial plasma pH and bicarbonate concentration, but not $P_{CO2}$ differed between control frogs (Table 1) and frogs from the feeding experiments.
This could be a seasonal effect, because the control experiments were performed in August and feeding experiments were from November to January. Seasonal effects on blood acid-base parameters have been previously reported in *Rana catesbeiana* even after weeks of acclimation at constant temperature (26).

Gastric acid secretion into the stomach after feeding induced a significant alkaline tide in arterial blood (Figs. 4 and 5). The acid-base status was composed of a large metabolic alkalosis (increase in bicarbonate), which was counteracted by a respiratory acidosis (increase in carbon dioxide tension). The increase in plasma \([\text{HCO}_3^-]\) was mirrored by a decrease in \([\text{Cl}^-]\) (Fig. 9), which probably is a consequence of active gastric H\(^+\) secretion by the H\(^+-K^+\)-ATPase followed by passive K\(^+\) and Cl\(^-\) diffusion (25). The respiratory compensation of the metabolic alkalosis was substantial, as pH would have increased by 0.18 units 48 h after feeding compared with the observed 0.08 units if \(\text{PCO}_2\) had remained constant (Fig. 5). An increase in \(\text{PaCO}_2\) during digestion was also suggested in *Bufo marinus* (36). This response most likely results from a reduction in ventilation relative to the rate of CO\(_2\) efflux.

Table 2. Acid-base parameters in sartorius muscle of the frog *Rana catesbeiana* before and 48 h after feeding

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>([\text{HCO}_3^-]), mM</th>
<th>(\text{PCO}_2), mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starved (control)</td>
<td>7.27±0.01</td>
<td>9.00±0.60</td>
<td>15.54±0.99</td>
</tr>
<tr>
<td>Fed (48 h)</td>
<td>7.32±0.02*</td>
<td>11.03±1.25</td>
<td>17.51±2.75</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; \(n = 4\) and 5 for starved and fed frogs, respectively. *Significantly different from corresponding control value \((P < 0.05)\).
production, although the possibility that cutaneous CO₂ conductance decreased during digestion cannot be dismissed. A similar response was also observed in pythons (unpublished observation), and, consistent with a relative hypoventilation, end-tidal P<sub>CO<sub>2</sub> increases during digestion in varanid lizards (11). Thus it appears that a respiratory compensation of the metabolic alkalosis may be a universal response among air-breathing ectotherms.

The respiratory compensation of the metabolic alkalosis seen in digesting frogs and pythons may limit inappropriate changes in blood oxygen affinity. pH in the RBCs was not measured in the present study, but the relative changes in RBC and plasma [HCO₃⁻] were similar (Fig. 4B), indicating that the plasma metabolic alkalosis was transferred to the RBCs. The rise in pH will increase the Hb oxygen affinity via the Bohr effect, which tends to reduce the unloading \( P_{O_2} \) in tissue capillaries, thus compromising tissue \( O_2 \) delivery in a situation where \( O_2 \) demand is actually increased and where a lowered \( O_2 \) affinity would be favorable (15). The respiratory acid-base compensation can therefore be viewed as a means to limit the unfavorable rise in oxygen affinity. In contrast to frogs and pythons, alligators develop a very large postprandial rise in pH (up to 0.3 pH units; Ref. 8) and apparently no respiratory compensation takes place (38). However, due to the unique allosteric binding of bicarbonate to Hb in alligators (2), the alkaline tide is expected to reduce the oxygen affinity and therefore improve oxygen unloading in the tissues.
The alkaline tide was also present in the intracellular space (Table 2), but the intracellular acid-base changes were small, suggesting that another reason for the postprandial hypercapnic acidosis could be protection of pH-sensitive metabolic pathways from the deleterious effect of a large alkalosis. Finally, the respiratory acidosis might play a role in gastric acid secretion, because an elevation of PCO₂ is supposed to speed up carbonic anhydrase catalyzed proton formation in parietal cells (31). Considering the above discussion, at least one important question remains. Why is the minor postprandial alkalosis not completely compensated? One reason could be that a (further?) reduction of the ventilation relative to the CO₂ production might cause a decrease in lung PO₂ that would compromise VO₂.

Between 48 and 72 h after feeding, plasma [HCO₃⁻] started to decrease in the bullfrog (Figs. 4B and 5). The decline in HCO₃⁻ levels may relate to increased base secretion to the intestine and decreased acid secretion to the stomach as food moves through the gastrointestinal tract.

The measured changes in acid-base parameters allow an estimate of the relative importance of the intracellular and extracellular spaces in providing protons for gastric HCl secretion. The estimate is presented in Table 4 and is based on the assumptions that the nonbicarbonate buffer value and the change in acid-base parameters in the sartorius muscle apply to all intracellular compartments. The blood nonbicarbonate buffer values were calculated from the relationship between Hct and buffer value provided by McDonald et al. (19) for Bufo.

Table 3. Metabolite concentrations in various organs of the frog Rana catesbeiana before feeding and 48 h after feeding

<table>
<thead>
<tr>
<th>Tissue</th>
<th>PCr</th>
<th>Cr</th>
<th>ATP</th>
<th>Lactate</th>
<th>Ammonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sartorius</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Starved (control)</td>
<td>23.78 ± 1.98</td>
<td>8.63 ± 0.76</td>
<td>5.38 ± 0.36</td>
<td>0.21 ± 0.13</td>
<td>0.16 ± 0.07</td>
</tr>
<tr>
<td>Fed (48 h)</td>
<td>18.84 ± 0.60*</td>
<td>8.46 ± 1.39</td>
<td>6.07 ± 0.39</td>
<td>0.16 ± 0.13</td>
<td>0.53 ± 0.11*</td>
</tr>
<tr>
<td>Ventricle</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Starved (control)</td>
<td>2.91 ± 0.16</td>
<td>2.61 ± 0.20</td>
<td>3.85 ± 0.26</td>
<td>0.15 ± 0.09</td>
<td>0.19 ± 0.11</td>
</tr>
<tr>
<td>Fed (48 h)</td>
<td>1.36 ± 0.41*</td>
<td>3.80 ± 0.62</td>
<td>4.01 ± 0.25</td>
<td>0.85 ± 0.52</td>
<td>0.59 ± 0.18</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starved (control)</td>
<td>ND</td>
<td>ND</td>
<td>2.27 ± 0.26</td>
<td>0.17 ± 0.08</td>
<td>3.12 ± 0.89</td>
</tr>
<tr>
<td>Fed (48 h)</td>
<td>ND</td>
<td>ND</td>
<td>2.40 ± 0.36</td>
<td>0.20 ± 0.07</td>
<td>6.05 ± 2.10</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE in μmol/g fresh wt of tissue; n = 4 and 5 for starved and fed animals, respectively. PCr, phosphocreatine; Cr, creatine; ND, not determined. *Significantly different from corresponding control value (P < 0.05).
The tissue water distribution, extracellular volume, and blood volume were adopted from Thorson (33). The increase in extra- and intracellular base excess after 48 h of digestion amounts to 3.4 and 2.1 mmol/kg animal, respectively, suggesting that both compartments provide protons for gastric acid secretion. Although nonsignificant, the change from net excess after 48 h of digestion may indicate that oxygen and/or metabolic stress, this may indicate that oxygen and/or metabolic stress, this may indicate that oxygen and/or metabolic stress, this may indicate that oxygen and/or metabolic stress, this may indicate that oxygen and/or metabolic stress, this may indicate that oxygen and/or metabolic stress, this may indicate that oxygen and/or metabolic stress, this may indicate that oxygen and/or metabolic stress, this may indicate that oxygen and/or metabolic stress, this may indicate that oxygen and/or metabolic stress. This response may help limit the oxygen affinity increase associated with a pH increase, which lowers the unloading oxygen pressure in the tissue capillaries. If this idea is correct, then a comparison with alligators is desirable, because their oxygen-linked binding of bicarbonate to Hb reduces the oxygen affinity during an alkaline tide. Metabolic data indicate that the intensity of the SDA response might be limited by the aerobic capacity of the heart. This could be a special feature in anuran amphibians, which lack coronary circulation and therefore depend on luminal oxygen delivery to the ventricle. Experiments on amphibians with and without coronary arteries may shed light on this possibility. It would also be interesting to see if VO2 reaches a plateau when the meal size is increased beyond what was used in this study.

Metabolite Status

Feeding caused a significant reduction in the phosphocreatine content in the sartorius muscle and ventricle, but ATP levels remained sufficiently buffered (buffer value). As phosphocreatine is a very sensitive indicator of metabolic stress, this may indicate that oxygen and/or substrate delivery to some tissues was compromised during digestion. High-energy phosphates displayed similar changes in the ventricle of Bufo marinus during long-term exposure to severe environmental hypoxia (24). In the anuran heart, which lacks a coronary circulation, a lowering of PO2 in systemic venous blood (resulting from a higher tissue oxygen extraction) may compromise luminal oxygen delivery to the ventricle and therefore contribute to limiting the intensity of the SDA response. In accordance with that, blood drawn from the ventricle of the snake Python molurus showed an 80% decrease in PO2 after feeding (27). The decrease in phosphocreatine levels was not mirrored by a significant accumulation in intracellular lactate (Table 3), which could be either due to a low production rate or a continued release to the blood with subsequent dilution, excretion, or metabolism by other organs (Fig. 10B).

Table 4. Changes in extra- and intracellular body fluid base excess after 48 h of digestion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Method of Calculation</th>
<th>Change, mmol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>( V_{blood} \times (\Delta[HCO_3^{-}]<em>{plasma} + [\beta</em>{NC,plasma} \times \Delta pH_{plasma} \times F_{CO2}] ) )</td>
<td>0.8</td>
</tr>
<tr>
<td>Interstitial fluid</td>
<td>( V_{inter} \times 1.05 \times \Delta[HCO_3^{-}]_{plasma} )</td>
<td>2.6</td>
</tr>
<tr>
<td>Intracellular fluid</td>
<td>( V_{intra} \times (\Delta[HCO_3^{-}]<em>{muscle} + [\beta</em>{NC,muscle} \times \Delta pH_{muscle} ] ) )</td>
<td>2.1</td>
</tr>
<tr>
<td>Total change in body fluids</td>
<td></td>
<td>5.5</td>
</tr>
</tbody>
</table>

\( F_{CO2} \) is ratio between total CO2 contents in blood and plasma; \( V_{blood} \), \( V_{inter} \), and \( V_{intra} \) are blood, interstitial fluid, and intracellular fluid volumes, respectively; \( \beta_{NC,plasma} \) is true plasma nonbicarbonate buffer value; \( \beta_{NC,muscle} \) is intracellular sartorius muscle nonbicarbonate buffer value.

Perspectives

The present study is the first integrative study of changes in metabolism, respiratory parameters, and acid-base status after feeding in a cannulated ectothermic vertebrate. Feeding was associated with a large increase in Hct, which appeared due to release of RBCs from the spleen. Further experiments (e.g., involving spleen ligation) would be informative in providing a definitive conclusion regarding the mechanism of the Hct increase and in evaluating its importance for safeguarding blood O2 transport during digestion. The extracellular metabolic alkalosis caused by gastric acid secretion after feeding was countered by an increase in PaCO2. This response may help limit the oxygen affinity increase associated with a pH increase, which lowers the unloading oxygen pressure in the tissue capillaries. If this idea is correct, then a comparison with alligators is desirable, because their oxygen-linked binding of bicarbonate to Hb reduces the oxygen affinity during an alkaline tide. Metabolic data indicate that the intensity of the SDA response might be limited by the aerobic capacity of the heart. This could be a special feature in anuran amphibians, which lack coronary circulation and therefore depend on luminal oxygen delivery to the ventricle. Experiments on amphibians with and without coronary arteries may shed light on this possibility. It would also be interesting to see if VO2 reaches a plateau when the meal size is increased beyond what was used in this study.

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