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 ~1/10 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 CARNIVOROUS VERTEBRATES habitually ingest very large meals that induce several-fold increases in oxygen uptake (VVO2). This response, often referred to as specific dynamic action (SDA), varies among species and depends on the size of the meal. In snakes, VO2 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 gas 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 Pco2 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 PO2 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

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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 digestion of big meals is a prolonged process, and 2 wk of starvation before experimentation, frogs were fed mice or rats equivalent to 10% of the body mass seems realistic. To our knowledge, there are no data for clearance rates of food after massive feeding in amphibians. However, as defecation did not start until after at least 5 days, digestion of big meals is a prolonged process, and 2 wk of starvation before experimentation appeared realistic.

Experimental Protocol

Series I: Oxygen consumption and carbon dioxide excretion. \( \dot{V}O_2 \) and carbon dioxide production (\( \dot{V}CO_2 \)) 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 thermostated 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 \( O_2 \) and \( CO_2 \) 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 (\( \dot{V}O_2 \) and \( \dot{V}CO_2 \)), 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 \( \dot{V}CO_2/\dot{V}O_2 \).

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 occlusively 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. Tetracyclinehydrochloride (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 reinfected 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.

Series III: Intracellular acid-base parameters, buffer values, and metabolite status. To reduce the number of animals needed for analysis of the influence of feeding on intracellular acid-base parameters and metabolites, catheterized frogs were divided into two groups only: 1) a control group (starved frogs) and 2) a 48-h postprandial group (frogs sampled 48 h after feeding). Sampling 48 h after feeding was chosen because the first two experimental series revealed substantial changes in both metabolic rate and plasma acid-base parameters at this time. Control frogs (395 ± 18, n = 4) were placed in the experimental chamber for at least 96 h, and a blood sample was drawn. Frogs in the other group (438 ± 36, n = 5) were placed in the experimental chamber for 48 h and then fed mice or rats (43 ± 4 g). Forty-eight hours after feeding, a blood sample was drawn. After blood sampling and analysis, frogs in both groups were anesthetized by adding a pH-neutralized solution of MS-222 (final concentration 3 g/l) to the animal chamber. After ~12–15 min, with very limited preceding activity, anesthesia was completed and the sartorius muscle, liver, and ventricle were quickly excised, freeze-dried, and stored in liquid nitrogen.

Analytic Procedures

Blood measurements. Arterial blood was analyzed for \( P_{\text{a}O_2} \) and pH using a Radiometer (Copenhagen, Denmark) \( O_2 \) electrode (ES5046–0) 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 \( O_2 \) (\( C_{\text{O}_2} \)) and \( CO_2 \) (\( C_{\text{CO}_2} \)) in whole blood and plasma were measured as described by Tucker (34) and Cameron (6) and corrected according to Bridges et al. (5). Arterial \( P_{\text{a}CO_2} \) (\( P_{\text{a}CO_2} \)) was calculated from the Henderson-Hasselbalch equation using \( pK_a\) and \( CO_2 \) solubility values (\( \alpha_{CO_2} \)) from Heisler (13). The plasma \( HCO_3^- \) concentration ([\( HCO_3^- \)]) was calculated as plasma \( C_{\text{CO}_2} - \alpha_{CO_2} \times P_{\text{a}CO_2} \). Hematocrit (Hct) was determined after 3 min centrifugation at 12,000 rpm in capillary tubes. The apparent RBC bicarbonate concentration ([\( HCO_3^- \text{RBC} \)]) was calculated from the plasma (pl) and whole blood (wb)
standard enzymatic tests after tissue extraction in 8% HClO4 and ATP) were analyzed in sartorius, ventricle, and liver by lites (lactate, total ammonium, creatine phosphate, creatine, equivalents \[HCO_3^-\]) was expressed per kilogram of RBC water, assuming a CCO2 and Hct using the formula \[[HCO_3^-]_{RBC} = \frac{\text{CCO}_2 - \alpha_{CO_2}}{\text{Hct}} \times \left[\text{CO}_2\right]_{sp} - \text{PCO}_2 \times \alpha_{CO_2}\] where \(\alpha_{CO_2}\) is the solubility of oxygen in human blood at 22°C (7). Hb concentration was measured spectrophotometrically after conversion to cyanmethemoglobin and application of a millimolar extinction coefficient of 11 (39). Methemoglobin (Hbmet) was measured according to the method of Benesch et al. (3). The fractional Hb oxygen saturation (HBO2-sat) 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 short, frozen tissue samples were ground under liquid nitrogen and metabolism was arrested using 130 mM potassium fluoride (KF) and 5 mM nitritolactetic acid (NTA), pH and total CO2 of the supernatant were measured as described above. Total CO2 was corrected for contamination with extracellular CO2 (23). Fractional tissue water content and extracellular water content of sartorius muscle were approximated by values for the triceps femorius and gracilis complex (38). Finally, intracellular [HCO3-] was determined in tissue homogenates as described by Poortner (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 KHCO3) 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. \(\alpha_{CO_2}\) was calculated according to Heisler (13), using an osmolyte of 850 mM (osmolality of the supernatant).

Transepithelial 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 (\(\Delta[\text{SID}]\)), 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+]) and in respiratory gas exchange ratio (RE; B) in Rana catesbeiana after a food intake of \(-\frac{1}{10}\) of their body weight. Values at time 0 are control values obtained just before feeding. Means ± SE; n = 5. *Significant difference from control values.

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

\(\text{V}O_2\) and \(\text{V}CO_2\)

The \(\text{V}O_2\) of fasting animals at rest was 27 ml O2·kg\(^{-1}\)·h\(^{-1}\) (Fig. 1A), which is equivalent to a standard metabolic rate of 0.54 kJ·kg\(^{-1}\)·h\(^{-1}\). After feeding, \(\text{V}O_2\) increased significantly, peaking at a value approximately three times higher than the predigestive value 96 h after feeding (Fig. 1A). \(\text{V}O_2\) remained significantly elevated above the time zero fasting value throughout the experimental period (Fig. 1A). \(\text{V}O_2\) more or less paralleled \(\text{V}CO_2\) (Fig. 1A), whereby feeding only induced

**Fig. 1.** Changes in oxygen uptake (\(\text{V}O_2\); ■) and carbon dioxide release (\(\text{V}CO_2\); ●) and in respiratory gas exchange ratio (RE; B) in Rana catesbeiana after a food intake of \(-\frac{1}{10}\) 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

$P_{O2}$ displayed a minor and nonsignificant decrease
after feeding (Fig. 2A), and $HbO_2$-sat (Fig. 2B) re-
ained virtually unaffected. Arterial $O_2$ content, how-
ever, was significantly increased throughout the experi-
mental period (Fig. 2C). This was due to a significant
increase in Hct (Fig. 3A) and hemoglobin concentra-
tion (Fig. 3B) with the mean cellular hemoglobin concen-
tration 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 ($P_{O2}$; A), oxygen satura-
tion ($HbO_2$; B), and oxygen content (C) in Rana catesbeiana after food
intake of $\frac{1}{10}$ of body weight. Means ± 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 $P_{CO2}$ 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 com-
pensated by a concomitant respiratory acidosis, result-
going in an only minor rise in pHi 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
metabolic alkalosis were present, is included for com-
parison (Fig. 5). At 96 h, pHi was back to the control...
Table 1. Blood and plasma acid-base, hematological, ionic, and metabolite status in true control (unfed) frogs

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
<th>120 h</th>
<th>144 h</th>
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<tbody>
<tr>
<td>pH</td>
<td>7.945 ± 0.014</td>
<td>7.966 ± 0.023</td>
<td>7.959 ± 0.026</td>
<td>7.962 ± 0.031</td>
<td>7.961 ± 0.034</td>
<td>7.893 ± 0.021</td>
<td>7.915 ± 0.028</td>
</tr>
<tr>
<td>HCO₃⁻, mM</td>
<td>30.3 ± 1.4</td>
<td>32.1 ± 0.5</td>
<td>30.9 ± 2.2</td>
<td>30.7 ± 2.0</td>
<td>31.0 ± 0.8</td>
<td>29.2 ± 1.8</td>
<td>31.3 ± 1.6</td>
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<tr>
<td>Pco₂, mmHg</td>
<td>10.7 ± 0.3</td>
<td>10.8 ± 0.7</td>
<td>10.6 ± 1.1</td>
<td>10.4 ± 0.4</td>
<td>10.6 ± 0.9</td>
<td>11.8 ± 0.1</td>
<td>11.9 ± 0.3</td>
</tr>
<tr>
<td>Po₂, mmHg</td>
<td>77.2 ± 3.9</td>
<td>86.7 ± 7.9</td>
<td>76.3 ± 4.8</td>
<td>81.3 ± 3.8</td>
<td>81.3 ± 5.1</td>
<td>78.0 ± 0.8</td>
<td>82.5 ± 2.5</td>
</tr>
<tr>
<td>O₂ content</td>
<td>1.29 ± 0.027</td>
<td>1.27 ± 0.17</td>
<td>1.64 ± 0.12</td>
<td>1.59 ± 0.19</td>
<td>1.23 ± 0.03</td>
<td>1.32 ± 0.09</td>
<td>1.40 ± 0.05</td>
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<tr>
<td>HbO₂ sat</td>
<td>75 ± 10</td>
<td>86 ± 5</td>
<td>84 ± 5</td>
<td>97 ± 11</td>
<td>94 ± 4</td>
<td>84 ± 1</td>
<td>86 ± 5</td>
</tr>
<tr>
<td>Hb, mM</td>
<td>1.51 ± 0.18</td>
<td>1.34 ± 0.23</td>
<td>1.83 ± 0.27</td>
<td>1.55 ± 0.27</td>
<td>1.17 ± 0.08</td>
<td>1.43 ± 0.13</td>
<td>1.49 ± 0.09</td>
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<tr>
<td>Hct</td>
<td>11.1 ± 1.5</td>
<td>12.0 ± 2.8</td>
<td>15.1 ± 1.5</td>
<td>13.0 ± 2.1</td>
<td>13.1 ± 2.6</td>
<td>13.9 ± 1.9</td>
<td>11.9 ± 0.5</td>
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<tr>
<td>MCHC</td>
<td>13.7 ± 0.5</td>
<td>13.3 ± 0.5</td>
<td>12.0 ± 0.6</td>
<td>11.9 ± 0.4</td>
<td>11.0 ± 0.5</td>
<td>11.7 ± 0.1</td>
<td>12.5 ± 0.3</td>
</tr>
<tr>
<td>Na⁺, mM</td>
<td>93.3 ± 3.5</td>
<td>94.7 ± 0.9</td>
<td>97.0 ± 2.1</td>
<td>97.5 ± 2.0</td>
<td>101.3 ± 2.2</td>
<td>96.3 ± 1.3</td>
<td>97.7 ± 3.4</td>
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<tr>
<td>Cl⁻, mM</td>
<td>52.3 ± 2.2</td>
<td>56.7 ± 1.5</td>
<td>64.2 ± 3.8</td>
<td>60.1 ± 0.6</td>
<td>63.0 ± 0.8</td>
<td>61.0 ± 1.1</td>
<td>63.1 ± 3.4</td>
</tr>
<tr>
<td>K⁺, mM</td>
<td>1.5 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.1</td>
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<tr>
<td>Osmolarity, mosmol</td>
<td>202 ± 1</td>
<td>196 ± 8</td>
<td>208 ± 7</td>
<td>190 ± 1</td>
<td>202 ± 4</td>
<td>192 ± 5</td>
<td>197 ± 7</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>0.8 ± 0.5</td>
<td>1.4 ± 1.0</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>Ammonia, mM</td>
<td>0.034 ± 0.018</td>
<td>0.016 ± 0.012</td>
<td>0.020 ± 0.018</td>
<td>0.061 ± 0.045</td>
<td>0.038 ± 0.018</td>
<td>0.042 ± 0.017</td>
<td>0.056 ± 0.036</td>
</tr>
<tr>
<td>Protein, g/l</td>
<td>57.8 ± 5.5</td>
<td>55.4 ± 4.8</td>
<td>63.1 ± 2.9</td>
<td>52.8 ± 1.3</td>
<td>57.0 ± 4.8</td>
<td>58.6 ± 8.8</td>
<td>55.2 ± 2.2</td>
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</tbody>
</table>

Data are presented as means ± SE; n = 3. HbO₂ sat, hemoglobin O₂ saturation; Hct, hematocrit; MCHC, mean cellular hemoglobin concentration.

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·pH unit⁻¹·kg⁻¹ tissue (n = 3) before feeding and 34.5 ± 1.4 mmol·pH 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 mmol·kg⁻¹·h⁻¹ before feeding to ~400 mmol·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 increase in phosphocreatine content 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 VCO₂ 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...
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

\( \text{Pa}_{\text{O}_2} \) and \( \text{HbO}_2\text{-sat} \) remained high during digestion (Fig. 2, A 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 \( \text{O}_2 \) levels relative to left atrial blood and most likely explains why \( \text{HbO}_2\text{-sat} \) was \( \approx 0.9 \) (Fig. 2B). The constant \( \text{HbO}_2\text{-sat} \) 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 \( \text{HbO}_2\text{-sat} \) during the postprandial period. In contrast to our study and the study of Wang et al. (36), Secor and Diamond (27) reported that blood \( \text{Po}_2 \) of pythons plunges to \( \approx 20 \text{ mmHg} \) during digestion. However, because blood samples were obtained by cardiac puncture, this value is unlikely to reflect arterial values in undisturbed animals.

The increased \( \text{Hct} \) (Fig. 3A) and \( \text{Hb} \) 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 \( \text{Bufo marinus} \). To our knowledge, there are no other reports on changes in hematological parameters in lower vertebrates after feeding. The increase in \( \text{Hct} \) 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 \( \text{Hct} \) observed in many fish species during hypoxia results, at least partly, from splenic contractions (14). However, the increase in \( \text{Hct} \) caused by hypoxic exposure in cold-submerged \( \text{Rana catesbeiana} \) 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 \( \text{Hb} \) 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 \( \text{Hct} \) and \( \text{Hb} \) concentration is due to a release of RBCs from the spleen to improve oxygen transport. In support of this, changes in \( \text{Hct} \) and \( \text{Hb} \) concentration and changes in metabolic rate showed similar time courses (Figs. 1A and 3A and B). Furthermore, the frogs with the lowest \( \text{Hct} \) values before feeding attained the highest \( \text{Hct} \) 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 \( \text{Hct} \) value correlates with depressed metabolic activities (including digestive activities). Thus a higher postprandial \( \dot{\text{V}} \text{O}_2 \) 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 \( \text{pH} \) and bicarbonate concentration, but not \( \text{PCO}_2 \), 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 [HCO₃⁻] was mirrored by a decrease in [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 PCO₂ had remained constant (Fig. 5). An increase in PaCO₂ during digestion was also suggested in *Bufo marinus* (36). This response most likely results from a reduction in ventilation relative to the rate of CO₂.

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>[HCO₃⁻], mM</th>
<th>PCO₂, 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 Pco₂ 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 Po₂ in tissue capillaries, thus compromising tissue O₂ delivery in a situation where O₂ demand is actually increased and where a lowered O₂ 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.

Fig. 7. Changes in plasma osmolality (A) and protein concentration (B) after feeding. Other details as in Fig. 2.

Fig. 8. Changes in plasma concentrations of Na⁺ (A), K⁺ (B), and Cl⁻ (C) after feeding. Other details as in Fig. 2. *Significant difference from time 0 predigestive values.

Fig. 9. Relationship between changes (Δ) in plasma [Cl⁻] and [HCO₃⁻] after feeding. Changes are calculated from data presented in Figs. 4B and 8C.
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 V'O₂.

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>
<td></td>
<td></td>
<td></td>
</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>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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).
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>$V_{\text{blood}} \times (\Delta[HCO_3^-]<em>{\text{plasma}} + \beta_N{C,plasma} \times \Delta PH</em>{\text{plasma}} \times F_{CO_2}$</td>
<td>0.8</td>
</tr>
<tr>
<td>Interstitial fluid</td>
<td>$V_{\text{inter}} \times 1.05 \times \Delta[HCO_3^-]$</td>
<td>2.6</td>
</tr>
<tr>
<td>Intracellular fluid</td>
<td>$V_{\text{intra}} \times (\Delta[HCO_3^-]<em>{\text{muscle}} + \beta_N{C,muscle} \times \Delta PH</em>{\text{muscle}})$</td>
<td>2.1</td>
</tr>
<tr>
<td>Total change in body fluids base excess</td>
<td></td>
<td>5.5</td>
</tr>
</tbody>
</table>

$F_{CO_2}$ is ratio between total CO2 contents in blood and plasma; $V_{\text{blood}}$, $V_{\text{inter}}$, and $V_{\text{intra}}$ are blood, interstitial fluid, and intracellular fluid volumes, respectively; $\beta_N{C,plasma}$ is true plasma nonbicarbonate buffer value; $\beta_N{C,muscle}$ is intracellular sartorius muscle nonbicarbonate buffer value.

marinus. 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 transepithelial acid excretion before feeding to a net acid uptake between 24 and 48 h after feeding (Fig. 6B) indicates that appropriate changes in extragastrointestinal transepithelial fluxes of acid-base equivalents may also contribute to acid secretion to the stomach. An estimate of the total proton secretion into the stomach is not, however, possible from the data in the present study, because proton turnover from metabolism is likely to change after feeding (21).

Metabolite Status

Feeding caused a significant reduction in the phosphocreatine content in the sartorius muscle and ventricle, but ATP levels remained sufficiently buffered (Table 3). 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).

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 definite 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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